Initiation of a pre-breeding programme for enhancing genetic resistance against wheat rust

by
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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2012

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Abstract

Plant diseases are among the major causes of food insecurity. In South Africa the wheat fungal diseases including stem rust caused by *Puccinia graminis* f. sp. *tritici*, leaf rust caused by *P. triticina* and stripe rust caused by *P. striiformis* f. sp. *tritici* are the most important. Genetic resistance is a viable way of protecting wheat crops against the wheat rusts, especially cultivars carrying multiple genes that confer durable resistance. In order to breed for multi-gene resistance an effective breeding strategy that allows for selecting multiple resistance genes and other desirable traits needs to be devised.

The aim of this study was to identify a number of genotypes with combinations of different rust resistance genes, good grain yield and end-use quality out of an existing pre-breeding population and thereby identify superior parents. In order to achieve the stated aim the following objectives have been identified: identify wheat lines through marker-assisted selection (MAS) carrying the gene complexes, *Sr31*/*Lr26*/*Yr9*, *Lr24*/*Sr24*, *Lr37*/*Sr38*/*Yr17*, *Lr34/Yr18* and *Sr2*; to develop inbred lines to evaluate selected lines under field trials.

From the initial subset of 64 lines, 60 were chosen and advanced to the doubled haploid (DH) phase and seed multiplication. The 60 lines either carried one or more of the three rust resistance gene complexes. The genes that were the most prominent were *Sr31*/*Lr26*/*Yr9* and *Lr24*/*Sr24*. The selected lines were incorporated into a DH seed multiplication phase. After 4 cycles of seed increases and preliminary field evaluation during multiplication, 15 lines were chosen and subjected to multi-location field trails.

The extensive multi-location field trails carried out in this study aided in identifying genotypes from the 15 MS-MARS lines with good adaptability and stability in regards to yield and baking quality. An important observation was that the molecular markers employed to indentify quality loci correlated well with the genes encoding the HMW-GS 5, 10 and 12 as observed with the Agilent© 2100 Bioanalyzer.

In future studies the lines which performed the best could be re-introduced into the existing MS-MARS pre-breeding programme of the Stellenbosch University's Plant Breeding Laboratory (SU-PBL). The frequencies of desired alleles could be increased in this manner. Since the majority of these characteristics are influenced by quantitatively inherited alleles, using these lines as recurrent

parents will increase the frequencies of these alleles in the existing SU-PBL pre-breeding population.

Uittreksel

Plantsiektes is van die belangrikste oorsake van voedselonsekerheid ter wêreld. In Suid-Afrika is die roesswamme van die belangrikste plantsiektes wat koring produksie beïnvloed. Hierdie siektes sluit in, stamroes wat veroorsaak word deur *Puccinia graminis* f. sp. *tritici*, blaarroes wat veroorsaak word deur *P. triticina* en streeproes wat veroorsaak word deur *P. striiformis* f. sp. *tritici*. Genetiese weerstand is ‘n uitstekende manier om koring te beskerm teen hierdie swamsiektes. Weerstand wat gebasseer is op veelvuldige weerstandsgene is veral ‘n goeie middel om genetiese-weerstand op ‘n volhoubare basis in koringteling toe te pas. Om veelvuldige weerstandsgene in koringkultivars in te teel word ‘n effektiewe telingstrategie benodig.

Die doel van die studie was om genotipes te identifiseer met kombinasies van veelvuldige weerstandsgene vir roes, sowel as goeie eienskappe belangrik vir graanopbrengs en bakkwaliteit. Lyne is geïdentifiseer uit ‘n bestaande voortelingspopulasie van Stellenbosch Universiteit se Planteteelt Laboratorium (SU-PTL) wat geteel was met spesifiek weerstand en opbrengs potensiaal in gedagte. Om die doel van die studie te bereik is sekere doelwitte daar gestel. Hierdie doelwitte sluit in om lyne uit die populasie te selekteer deur middel van merker bemiddelde seleksie (MBS) vir gene naamlik *Sr31*/*Lr26*/*Yr9*, *Lr24*/*Sr24*, *Lr37*/*Sr38*/*Yr17*, *Lr34/Yr18* en *Sr2*; om die geselekteerde lyne suiwertelend te maak; sowel as om die suiwertelende lyne in veld proewe in te sluit.

Van die oorspronklike stel van 64 lyne, is 60 gekies vir verdere studie. Deur middel van die verdubbelde haploïed (VH) tegniek is die lyne suiwertelend gemaak. Die 60 lyne het een of meer van die geselekteerde gene bevat. Die mees prominente gene was die twee geen komplekse *Sr31*/*Lr26*/*Yr9* en *Lr24*/*Sr24*. Na vier siklusse van saadvermeerdering en voorloppige seleksies is 15 lyne ingesluit by ‘n multi-omgewing veldproef.

Hierdie uitgebreide multi-omgewing veldproewe het gehelp om individue uit die 15 lyne te identifiseer wat oor goeie aanpasbaarheid en stabiliteit beskik met betrekking tot opbrengs en bak kwaliteit. Die molekulêre merkers gebruik om die gene verantwoordelik vir die kodering van HMG-GS 5, 10 en 12 op te spoor het goed gekorreleer met die HMG-GS bande bepaal met behulp van die Agilent© 2100 Bioanalyzer.

Toekomstige studies kan moontlik insluit die gebruik van die lyne wat geïdentifiseer was met goeie kenmerke in die bestaande MS-MARS teelprogram van die SU-PTL. Die frekwensies van die verlangde allele kan op hierdie manier in die populasie verhoog word.

List of abbreviations

(Pty) Ltd	proprietary limited
°C	degrees celsius
2.4-D	2,4-dichlorophenoxyacetic acid
∞	infinity
AACC	American Association of Cereal Chemists
ABS	flour water absorption
AFLPs	amplified fragment length polymorphisms
AMMI	additive main effects and multiplicative interaction
Amp	marker amplification
ANOVA	analysis of variance
ARC-SGI	Agricultural Research Council-Small Grain Institute
avr-gene	avirulence gene
BFY	break flour yield
BME	beta-mercaptoethanol
bp	base pairs
CAPS	cleaved amplified polymorphic sequences
CIMMYT	Centro International de Mejoramiento de Maiz y Trigo (*International Maize and Wheat Improvement Centre*)
cM	centi Morgan
cm	centimetre
CO_2	carbon dioxide

CTAB	N-cetyl-N, N, N-trimethyl-ammonium bromide
CV	coefficient of variation
ddH_2O	double distilled autoclaved water
DIAM	seed diameter
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
EX	total flour extraction
FP	forward primer
f. sp.	formae specialis
FN	falling number
g	gram
GA	gibberilic acid
gDNA	genomic DNA
GEI	genotype by environment interaction
GLM	general linear model
Glu-1	Glutenin 1
H^2	broad senseheritability
H_2O	water
ha	hectare
HI	seed hardness
HLM	hectolitre mass

HMW-GS	high molecular weight glutenin subunit
Hz	hertz
IPCA	interaction principal component axes
$KBrO_3$	potassium bromate
kg	kilogram
kg/ha	kilogram per hectare
kg/hl	kilogram per hectolitre
L	litre
LMW-GS	low molecular weight glutenin subunit
LR	leaf rust
Lr	leaf rust resistance gene
LSD	least significant difference
LTN	leaf tip necrosis
m	metre
M	molar
MAS	marker-assisted selection
mg	milligram
Mg^{2+}	magnesium
min	minutes
ml	millilitre
mm	millimeter
mM	millimolar

MR	moderately resistant
MS	moderately susceptible
MSE_a	error mean squares from the NNA ANOVA
MSE_u	error mean squares from the GLM ANOVA
MS-MARS	male sterility mediated marker-assisted recurrent selection
NAOAc	sodium acetate
ng	nanogram
NNA	nearest neighbour analysis
PBC	pseudo black chaff
PCA	principal component analysis
PCR	polymerase chain reaction
Ppd-genes	photoperiod genes
PROT	protein content
PT	dough mixing time
R	resistant
RP	reverse primer
R^2	coefficient of determination
RAPDs	random amplified polymorphic DNAs
RFLPs	restriction fragment length polymorphisms
R-gene	resistance gene
Rht-genes	reduced height genes
RMS	recurrent mass selection

RNase A	Ribonuclease A
rpm	revolutions per minute
S	susceptible
SAGL	South African Grain Laboratory
SCAR	sequenced characterized amplified region
SD	standard deviation
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
sec	seconds
Sec-1	secalin 1
SKCS	single kernel characterization system
SNPs	single nucleotide polymorphisms
spp.	species pluralis
SR	stem rust
Sr	stem rust resistance gene
SSD	single seed descent
SSE_a	error sum of squares from the NNA ANOVA
SSE_u	error sum of squares from the GLM ANOVA
SSR	simple-sequence repeat
STSs	sequence tagged sites
SU-PBL	Stellenbosch University Plant Breeding Laboratory
TKM	thousand kernel mass
ton/ha	ton per hectare

Tris-Cl	tris-chloride
Tris-EDTA	tris- ethylenediaminetetraacetic acid
U	unit
UVPgt	Universiteit Vrystaat *Puccinia graminis* f.sp. *tritici*
UVPrt	Universiteit Vrystaat *Puccinia recondita* f.sp. *tritici*
var.	variation
viz.	*videlicet*
VOL	bread volume
YR	stripe rust
Yr	stripe rust resistance gene
µg	microgram
µl	microlitre
µm	micrometer

List of figures

List of tables

Contents

1. Introduction

The global wheat demand is projected to increase by 60% to 880 million tons by 2050 (Singh *et al*., 2011). The challenge of reaching the 2050 global production needs is further complicated due to the fact that agricultural land used for food production is not increasing as the population is increasing, and that climate change is expected to reduce wheat production significantly in developing countries (Trethowan *et al*., 2007; Borlaug, 2007).

Breeding is one of the primary mechanisms to improve wheat cultivars to meet the predicted global demands (Fischer & Edmeades, 2010). The importance of wheat breeding is highlighted when realising that high yielding wheat cultivars should be developed that can withstand today's important wheat diseases, the predicted changes climate conditions, and still be able to produce enough food to feed the growing world population (Fischer & Edmeades, 2010). The first high-yielding semi dwarf wheat cultivar was released in the 1960's (Borlaug, 2007). Due to the effective utilization of these short straw wheat cultivars, world wheat yields more than doubled from 1960 to 1990 (Khush, 1999). This event came to be known as the Green Revolution (Borlaug, 2007). Despite the initial successes of the Green Revolution, the growth in grain yields is now starting to decline (Fischer & Edmeades, 2010; FAOSTAT, 2012). World wheat yields yearly increased by 0.9% over the period 1987 to 2007, this in contrast to the linear rates of yield increases per year of 1.03% for the period from 1961-1987 (Fischer & Edmeades, 2010; FAOSTAT 2012). With today's 0.9% yearly yield increases, the goal of achieving a 60% global wheat increase by 2050 would not be realised (Tweeten & Thompson, 2008). In all honesty a second Green Revolution is inevitable and improvement in genetic yield potential needs to drastically pick up pace in order to sustain the global growth in population (Reynolds *et al*., 2009).

Due to the destructiveness of plant diseases, it plays an integral role when it comes to food security (Singh *et al*. 2011). These diseases present itself in the form of fungi, bacteria and viruses (Bockus *et al*. 2009). In South Africa the wheat fungal diseases including stem rust caused by *Puccinia graminis* f. sp. *tritici*, leaf rust caused by *P. triticina* and stripe rust caused by *P. striiformis* f. sp. *tritici* are the most important wheat diseases that needs to be taken into consideration in wheat variental development (Pretorius *et al*., 2007).

Cultivars carrying multiple genes for resistance can contribute to sustainable wheat production due to their robust resistance against a broad-spectrum of diseases (Bariana *et al*., 2007). In order to breed for multi-gene resistance in wheat, a good breeding strategy that allows for selecting multiple

resistance genes needs to be in place (Marais & Botes, 2009). Marker-assisted selection (MAS) facilitates this, and gives the opportunity to select not only for one, but a wide array of genes in a single genotype (Mago *et al*., 2005).

In conventional wheat breeding resistance genes are fixed through inbreeding after crosses with desirable parents. In self-pollinators after each successive inbreeding generation heterozygosity is halved, because of the self-pollinating nature of wheat, making a large number of crosses is very difficult. When looking at multi-gene resistance in wheat, it can be very difficult to incorporate a number of different disease resistant genes in one genotype after only one single crossing cycle (Marais & Botes, 2009).

For the genetic improvement and allele enrichment of multi-traits in cross-pollinating species, the well-established breeding technique of recurrent mass selection (RMS) has proved its worth. This technique was primarily developed for the improvement of quantitatively inherited traits that is controlled by numerous genes. RMS is an equal applicable breeding strategy for self-pollinating crops such as wheat. The only difference comes in the reduced number of crossing combinations that can be performed on wheat compared to cross-pollinating crops such as maize.

One way of facilitating RMS in wheat is by using male sterility. Marais *et al*.(2000) introducedthe dominant male sterility gene *Ms3* (located on the short arm of chromosome 5A) from a winter wheat accession KS87UP9 into the spring wheat ‘Inia 66’. The progeny obtained from this cross segregated for the dominant *Ms3* gene. Male sterile lines were selected out of the progeny and used in a crossing scheme with male fertile wheat lines in order to facilitate large amount of different crossing combinations. This technique created a means of cross pollination in wheat and subsequently the implementation of a RMS scheme in wheat (Marais *et al*., 2000). With the implementation of MAS, wheat lines which has been ear marked for incorporating into the mass crossings, can be scrutinized on grounds of the genes each carries. When this is done prior to flowering, only the selected lines take part in the mass crossings. By allowing only wheat lines with sought after genes to cross with each other in successive cycles, allele enrichment and breeding for multi-gene resistance in wheat can be facilitated (Marais & Botes, 2009). This technique came to be known as male sterility mediated marker-assisted recurrent selection (MS-MARS).

Diversifying a wheat breeding population by incorporating a wide array of different genes is important. By doing this the genetic diversity is increased which helps in developing a more robust and durable breeding population (Reif *et al*., 2005). The high bread making quality standards of

South Africa, bestow strict quality guidelines upon newly developed wheat cultivars (Van Lill & Purchase, 1995). These guidelines forced breeders to breed wheat cultivars that need to adhere to these strict guidelines. In order to breed for quality, breeders often relied very heavily on a single recurrent quality donating parent to acquire these quality characteristics in their breeding populations. In accordance a decrease in wheat genetic diversity took place. The decrease of diversity was a direct result of actively utilizing and selecting only for specific wheat phenotypes (Reif *et al*., 2005).

The aim of this study was to identify a number of genotypes with combinations of different rust resistance, good grain yield and end-use quality characteristics out of an existing MS-MARS population of the Stellenbosch University Plant Breeding Laboratory (SU-PBL). This was done to identify superior crossing parents which could be used to further improve wheat breeding populations.

In order to achieve the stated aim the following objectives have been identified:

a. Identification of wheat lines from the MS-MARS scheme with the following multi-gene complexes present *Sr31*/*Lr26*/*Yr9*, *Lr24*/*Sr24*, *Lr37*/*Sr38*/*Yr17*, *Lr34/Yr18* and *Sr2*;
b. Development of inbred lines from material containing some or all of the genes mentioned above;
c. Perform field trials to evaluate the MS-MARS lines' agronomical and industrial attributes as well as their adult plant resistance to all three rusts.

2. Literature review

Farming begun about 12,000 years ago in the region commonly referred to as the Fertile Crescent (Moore *et al*., 2000). This region today envelopes modern day Israel, Jordan, Lebanon and western Syria, into south-east Turkey and, along the Tigris and Euphrates rivers, into Iraq and the western flanks of Iran (Zohary & Hopf, 2000). The development of farming happened after early human society discovered the nutritional value of certain wild animals they hunted and plants which they gathered (Gopher *et al*., 2002). With these discoveries they started domesticating the plants and animals for sustained survival (Salamini *et al*., 2002). This domestication led to the occurrence of selection, where the early farmers selected plants and animals that were acceptable to them according to their perception of the perfect phenotype (Gopher *et al*., 2002).

Grasses from the family *Poaceae* were some of the first plants domesticated by early human societies. This grass family contributed to the diverse general plant group that contains domesticated maize, rice, sorghum and wheat (Reif *et al*., 2005). These crops are responsible for most of the world's food resources, and even influence world politics due to the importance of the production and distribution thereof (Salem *et al*., 2007). Of the more important species from the *Poaceae* family is the *Triticum* species which had it's origin about 130 million years ago (Porceddu *et al*., 1988).

2.1. Wheat

Today wheat is cultivated worldwide as staple food with a global production estimated at 651 million tons for 2010/2011 (FAOSTAT, 2012). With an average yearly production of 2.02 million tons (2002 to 2011) wheat is one of the most important cultivated grain crops in South Africa (GRAIN SA, 2012).Wheat is being cultivated over all the climatic regions of South Africa. The two general wheat types that are grown over these different regions are winter and spring wheat. Winter wheat requires an extended exposure to cold temperatures to induce flowering, a process called vernalization. Spring wheat, however, does not have such requirements. Typical vernalization conditions are 4 to 6 weeks at less than 10°C. The need for vernalization is an adaptation of several cereal crops, such as wheat, barley, rye and oats, to cold climates (Yan *et al*., 2004). In winter wheat production regions vernalization is needed to regulate the onset of flowering. This is a sensible

adaptation that prevents the risk of flowering in winter, a time when freezing temperatures may inhibit seed production (Glover, 2007).

In South Africa wheat is cultivated in three macro environments namely the winter rainfall dryland production region, the summer rainfall dryland production region and the summer rainfall irrigation region. Winter wheat is cultivated in the summer rainfall dryland production region while spring wheat is cultivated in both the winter rainfall dryland production region and the summer rainfall irrigation region (Kilian, 2012). For these diverse environments breeding companies strategically focus on the different climatic conditions, biotic and abiotic stresses of the environments to breed cultivars adapted to each respectively (F.P. Koekemoer, personal communication, 2011).

2.1.1. Genetics

Bread wheat (*Triticum aestivum* L) is the product of a stable natural inter-species hybridization (Jauhar, 1992). This natural hybridization occurred in such a manner that developed wheat as a hexaploid possessing all three genomes from it's wild progenitors. These three genomes include the A-, B- and D contributed by *Triticum urartu*, *Aegilops speltoides* and *Aegilops tauschii*, respectively (Dvořák, 1976; Riley *et al*., 1958). The first complex hybridization of wheat was the hybridization between *T. urartu*, the donor of the A-genome (Dvořák, 1976) and *Ae. speltoides*, the donor of the B-genome (Riley *et al*., 1958). This hybridization was the origin of the tetraploid wild emmer wheat, *Triticum dicoccoides* (Kimber & Feldman, 1987). It is postulated that the tetraploid nature of wild emmer wheat (AABB) occurred by means of somatic chromosome doubling after the hybridization between *T. urartu*, the A-genome donor, and *Ae. speltoides*, the B-genome donor; or by means of meiotic non-reduction (Jauhar, 2006). It was further suggested that due to the tetraploid nature of *T. dicoccoides*, it had superior adaptation to warm drought stricken areas, which made it very attractive for cultivation by early farmers in all arid regions of the Fertile Crescent (Wilcox, 1995). These tetraploids also possessed hull-less seeds and soft glumes that helped in the harvesting process (Salamini *et al*., 2002). With the domestication of *T. dicoccoides* farmers continuously selected for better harvesting characteristics, which ultimately allowed the man driven evolution of free-threshing forms (Reif *et al*., 2005). The first free-threshing tetraploids were *T. turgidum* var. *dicoccoides* which are known today as durum wheat. During further cultivation, another spontaneous hybridization occurred between the tetraploid *T. turgidum* var. *dicoccoides* (AABB) and the diploid wild grass specie *Ae. tauschii* (DD) (McFadden & Sears, 1946). This subsequent

event gave rise to today's hexaploid bread wheat, *T. aestivum* (McFadden & Sears, 1946). The polyploidy nature of hexaploid bread wheat was of evolutionary importance mainly because it has facilitated the formation of a superstructure that combines various genetic materials of isolated diploids. The evolutionary advantage of polyploids over diploids is reflected in their very wide morphological and ecological variation (Feldman, 2001).

2.1.2. Breeding

Throughout early domestication and cultivation selection played an integral role in the development of modern high yielding hexaploid wheats (Salamini *et al*., 2002). Selection practiced by early farmers are seen by many wheat researchers as the origin of modern plant breeding (Gopher *et al*., 2002; Koornneef & Stam, 2001; Wilcox, 1995). From the first selections of improving primitive wheat cultivars to today's new and improved cultivars, wheat productivity changed significantly. One of the biggest changes wheat went through was during the Green Revolution (Trethowan *et al*., 2007). The 1960s were classified by most as a decade of food insecurity due to the problems the world faced in coping with the food and population balance (Khush, 1999). This imbalance was directly influenced by the acceleration in the growth of the population and the decline of arable farmland for food production. The acceleration of population growth was the direct effect of a decline in the overall human mortality rates influenced by advancements in modern medicine and health care (Khush, 1999). The decline in arable farmland was caused by mismanagement and over exploitation of virgin soil (Khush, 1999). With the challenges that were faced, international organizations and concerned professionals came together to raise awareness regarding the ensuing food crisis, and to mobilize global resources to help in addressing this global problem (Borlaug, 2007). Enough food needed to be produced for an ever increasing population on stagnating cultivation area (Borlaug, 2007). The focus was placed onto improving the two major cereal crops, wheat and rice (Evenson & Gollin, 2003; Khush, 1999). By the mid-1960s, scientists developed improved wheat and rice cultivars that were subsequently released to farmers in Latin America and Asia (Evenson & Gollin, 2003). The success was achieved through the use of improved cultivars, irrigation, fertilizer and crop protection measures and was characterized as the "Green Revolution" (Evenson & Gollin, 2003). The Green Revolution through wheat production was the direct effect of certain characteristics bred into the predominantly long straw wheat cultivars of the 1950s (Trethowan *et al*., 2007). These characteristics were visualized as shorter plant heights, influenced

by the *Rht*-genes, and the inability of photoperiod responsiveness, influenced by the *Ppd*-genes (Trethowan *et al*., 2007). These short statured-, or semi-dwarf cultivars, were more tolerant to lodging; they also partitioned more of the total biomass to the grain and subsequently had a higher harvest index. Owing to these traits, the semi-dwarf wheat cultivars had higher yield than the traditional long straw cultivars, especially when high levels of fertilizer were applied (Trethowan *et al*., 2007). Photoperiod insensitivity was introduced by using a shuttle-breeding scheme via selection of segregating generations between two contrasting environments; *viz.* shorter day length low altitude and longer day length and high altitude (Borlaug, 1968). These new photoperiod insensitive cultivars could have been planted any time of the year. Moreover, with the growth duration reduced it gave farmers the ability to plant two crops a year in some environments (Evenson & Gollin, 2003).

These fundamental changes together with improved disease resistance significantly enhanced the yield potential and stability of wheat in all the major wheat production regions (Sayre *et al*., 1997). Since the initial introduction of the new and improved semi-dwarf wheat cultivars, wheat yields have continued to improve at an average rate of 1% per year (Byerlee & Moya, 1993). A significant proportion of this improvement in yield have been found to be attributed to improvements in agronomic techniques; however it was also found that the majority of the average 1% per year gain in grain yield was the direct result of plant breeding (Bell *et al*., 1995).

By replacing the more traditional cultivars with the improved wheat cultivars, together with associated improvement in farm management practices, had a dramatic effect on total wheat production. Since 1966, when the first high-yielding wheat cultivar was released, the wheat area harvested increased only marginally while world wheat production increased from 308 million tons in 1966 to 541 million tons in 1990 (Khush, 1999). The biggest outcome of the Green Revolution was experienced in Asia where wheat production increased from 33 million tons in 1966 to 225 million tons in 1995. This outcome is visualized as a six fold increase in wheat production in a period of only 30-years (Khush, 1999). In contrast to the initial success phase of the Green Revolution, which showed world grain yields more than doubling from 1960 to1990 (Khush, 1999), the growth in grain yields is now declining (Fischer & Edmeades, 2010). Over a period from 1987 to 2007 the linear rates of yield increases for world wheat were estimated as 0.90% per year, this in contrast to the linear rates of yield increases of 1.03% for the period from 1961-1987 (Fischer & Edmeades, 2010; FAOSTAT 2012). Even if this relative rate of 0.90% increase in grain per year could be maintained, various studies suggest this would not prevent real food shortages, in the face

of projected demand growth to 2050 (Tweeten & Thompson, 2008). With these predictions, improvement in genetic yield potential needs to pick up pace in order to avoid added utilization of natural landscapes and the over intensification of current agro-ecosystems (Reynolds *et al*., 2009). Two other factors reinforce the need for genetic interventions to boost crop production; these are climate change and environmental concerns associated with intensive agriculture (Neelin *et al*., 2006; Montgomery, 2007). Climate change makes agricultural productivity less predictable and concerns about the environment highlights the need to develop more input-use efficient cropping systems (Reynolds *et al*., 2009). Increasing the genetic yield potential as well as wheat's inherent disease resistance may contribute to solving both these problems (Reynolds *et al*., 2009).

2.1.2.1. Breeding for agronomical characteristics

Fundamental wheat breeding together with new advancements in agricultural has made significant progress in increasing wheat yields (Bertrand *et al*., 2008). In order to breed better adapted cultivars, breeders need to constantly respond to changing agricultural practices, environmental conditions and consumer preferences. Changes in agricultural practices such as conservation tillage, a practice that is currently becoming more attractive due to input costs and environmental factors, needs to be addressed when breeding new wheat cultivars that can be adapted to these and other practices. Some of the characteristics to be bred into wheat for conservation tillage environments include allelopathy, which will give wheat cultivars better advantage over weeds, and longer coleoptile length, which will give cultivars better emergence capabilities through residues (Bertrand *et al*., 2008; Rebetzke *et al*., 2005).

With climate change taking effect, higher night and day temperatures are more frequently being recorded. To address these issues wheat breeders need to work hand in hand with wheat physiologists to look at characteristics that can be improved in wheat so that it can be more adapted to these increased temperature conditions. One such characteristic being looked at is canopy temperature depression which can be screened under normal as well as heat stressed conditions. Under both conditions cooler canopies means that stomatas are open and transpiration is freely taking place that is a carbon fixation process. High canopy temperature means stomata are closed and that respiration is taking place at a higher level than transpiration. This entails that energy is being used and that carbon fixation is not taking place. Cultivars with cooler canopy temperature inherently have better yields than their hotter canopy counter parts. Breeding for cooler canopy

temperature cultivars could help in sustaining wheat yield under increased temperature conditions (Reynolds *et al*., 2009).

Climate change also has a direct or indirect effect on both host plant resistance expression and on the pathogen's infection ability (Gregory *et al*., 2009). Elevation in temperature and the concentration of CO_2 is predicted to have an increased response in plant biomass that will ultimately increase the area under which plant pathogens can infect and spread from (Diaz *et al*., 1993; Gregory *et al*., 2009). Due to the increase of biomass, resistance genes must be utilized in an integrated manner to render these increased biomass area less viable for plant pathogens to colonization and proliferation on. It must also be taken into consideration that elevated levels of both ozone and CO_2 can affect expression of resistance genes in host plants (Plazek *et al*., 2001; Plessl *et al*., 2005). The majority of rust resistance genes, especially leaf and stripe rust, are temperature sensitive where they are either effective at low to moderate temperatures and not at high temperatures (McIntosh *et al*., 1995; Gregory *et al*., 2009).

Breeding for disease resistance based on genetic principles was initiated soon after the rediscovery of Mendel's laws in 1900, when it was demonstrated that resistance to yellow rust in wheat was controlled by a single recessive gene pair (Biffen, 1905). New and virulent pathotypes continuously evolving are that breaks resistance conditioned by major genes in a relative short amount of time, making resistance breeding a very important component of wheat breeding (Sawhney, 1995). Breeding for resistance thus needs to be focused in an anticipatory or pre emptive manner (Knott, 1989). To develop resistant wheat cultivars it is important to know the diversity of pathotypes. Continues rust surveys goes hand in hand with breeding for resistance because the breeder needs to know what to breed against (Pretorius *et al*., 2007). Deployment of diverse genetic sources for disease resistance in high-yielding wheats assists in achieving yield stability at higher levels of productivity, without resorting to costly chemicals (Sawhney, 1995).

Advances in wheat rust breeding depend on three prerequisites. Firstly, availability of new sources of genetic variation to provide desirable alleles; secondly, presence of technologies to recombine this variation into the generation of new genotypes; and thirdly, availability of technologies for identifying and selecting the phenotypes associated with the new adapted gene complexes.

2.1.2.2. Breeding for wheat quality

Wheat and wheat products provide some of the most easily acquired sources of energy and protein, which makes wheat an extremely important food source for humankind (Evans & Peacock, 1981; Salem *et al*., 2007). There are broadly two classes of wheat, hard wheat and soft wheat. Hard wheat, has a hard endosperm and is being used for raised or leaved bread, and is also satisfactory for steamed breads such as “chapattis”, tortillas” and “pieta”. Soft wheat, has a softer endosperm compared to hard wheat, and is primarily used for cakes, pastries, flat bread and crackers (Atwell, 2001).

Bread making is one of humankind’s oldest biotechnological processes, being established some 4000 years ago (Shewry *et al*., 1995; Goesaert *et al*., 2005). Due to the importance of wheat as food source in the world, quality plays a significant role in the development of new wheat cultivars.

With increased wheat production during the onset of the Green Revolution, major wheat import countries experienced a production shift to such an extent that they became net export countries (Trethowan *et al*., 2007). These countries now have aspirations of exporting wheat, and to compete in the global grain market making quality paramount. At the inception of the Green Revolution, the primary objectives of wheat breeders were higher, more stable yields. Internationally the use of high yielding semi-dwarf wheat cultivars led to major increases in grain yield but reduced grain protein concentration (Ortiz-Monasterio *et al*., 1997). The reduced grain protein percentage in these high yielding wheats was likely due to a negative correlation between these two trait’s, which arisen from a simple dilution effect of a given amount of protein in a sea of carbohydrates (Trethowan *et al*., 2007). Interestingly, during the same era, a study was conducted on baking quality of South African wheat cultivars spanning from 1930 to 1990. What was found was that wheat yield increased by 87% and baking quality by 20%. The increase of quality in the face of greatly increased yield was a significant genetic achievement. Around 47 years ago the bread making quality of South African wheat was of such poor standard that good quality wheat had to be imported to supplement local wheat. Since then wheat quality became an important factor in South African wheat breeding programmes (Van Lill & Purchase, 1995).

Protein in wheat grains mainly comprises of albumin, globulin, gliadin, and glutenin (Osborne, 1907). The albumin and globulin, known as the metabolism proteins, play a minor role in bread making quality while gliadin and glutenin, which together with water and physical energy form

gluten, plays a very important role. Gluten is the determinant factor to flour processing quality (Pang *et al*., 2009).

As advances in wheat research and specifically grain quality were made, the understanding of the genetic control of wheat quality also improved. The influence of the high- and low molecular weight glutenin subunit's (HMW-GS & LMW-GS) on protein quality and dough rheology has been explored and understood (Dumar *et al*., 2010; Payne, 1987; Pang *et al*., 2009). The contribution of HMW-GS to the processing quality can range from 35 to 40% (Pang *et al*., 2009).

The ability to make bread depends largely on the visco-elastic properties conferred to wheat doughs by the gluten proteins (Shewry *et al*., 1995). Wheat gluten is largely responsible for the functional properties of dough which allow the entrapment of CO_2 released by the yeast, giving rise to a light porous structure (Shewry *et al*., 1995). The gliadin component of gluten is largely responsible for gluten viscosity and the glutenin for it's elasticity (Shewry *et al*., 1995).

High-molecular-weight glutenin subunits are encoded by the *Glu-1* loci which are located on the long arms of chromosomes 1A, 1B, and 1D. These loci are designated as *Glu-A1*, *Glu-B1* and *Glu-D1* and correspond with their chromosomal location (Lawrence *et al*., 1981; Payne *et al*., 1982). Each locus consists of two genes encoding a low molecular weight x-typesubunit and a high molecular weight y-typesubunit (Shewry *et al*., 1992; Pang *et al*., 2009). In theory hexaploid wheat could contain six different subunit's, but in reality, due to silencing of some genes only three, four or five are present in cultivars of bread wheat (Shewry *et al*., 1992). Each of the *Glu-1* has different alleles of it's genes and for each allele a different subunit is encoded. Strong associations have been found between bread-making quality and these alleles, more so for the alleles at *Glu-D1* and *Glu-A1* loci. In most studies, *Glu-D1* is believed to be the largest contributor to processing quality at the *Glu-1* loci (Payne, 1987).

With present emphasis being placed on the molecular control of wheat quality the older traditional techniques of measuring dough rheology are still important and implemented. While the molecular techniques try to explain quality on the molecular level, the rheological techniques explain it on a physical level (Kent & Evers, 1994). Rheological measurements determine the macroscopic viscoelastic properties of dough and that of it's components (Kent & Evers, 1994). The most important tests being implemented in the South African wheat industry include the Farinograph (D'Appolonia & Kunerth, 1984), the Alveograph, the Mixograph, the Falling Number and the baking test. These tests determine a cultivar of dough characteristics; amongst others extensibility,

elasticity, optimum dough mixing time, stability to over mixing, α-amylase content, and ultimately the ability to bake a bread.

In the South African wheat-delivering network, wheat quality plays a pivotal role when it comes to delivering the grain at silo level. Grain quality can mean the difference between a class B1 (best wheat grading class) and a class B4 or other (feed quality). Of these the B1 grade is a much more economical sought after grade than B4. These grades of each batch being delivered are ascertained through physical screening and testing of the grain and flour. Some of the characteristics, which are looked at during delivering at the silos, include measurement of test weight, protein content and falling number. These characteristics are important to the miller that requires optimum flour yield, while good baking performance is important to the baker to provide acceptable end products to the consumer (Goesaert *et al*., 2005). For this reason breeders are constantly aspiring to improve the quality of newly developed cultivars.

2.2. Wheat rust

Wheat rust diseases are one of the major biotic factors limiting wheat production in South Africa, and the world. Several thousands of fungi attack a wide range of higher plants. A number of them cause serious economic losses in crops, but none more so than the three *Puccinia* spp. that attack wheat (Kolmer, 2005). *Puccinia* spp. have affected wheat for thousands of years and references of wheat rust can be found throughout literature in classical Greek and Roman civilization (Bolton *et al*., 2008).

The three wheat rust pathogens are stem rust, leaf rust and stripe rust, respectively caused by the pathogens *Puccinia graminis* Pers. f. sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici* (Pretorius *et al*., 2007; Singh *et al*., 2011). Although present day epidemics are not as frequent due to improved farming practices and more focused and structured wheat breeding programs, rusts still cause significant crop losses under favorable conditions (Singh *et al*., 2011).

In South Africa the rusts on wheat is mainly controlled by fungicide applications (Boshoff *et al*., 2002). These applications can vary from one to three times in a single wheat cropping season (Paul, 2009). This is a costly and potentially environmental unfriendly practise that can have a long term negative effect on wheat production in South Africa. For this reason breeding for rust resistance need to play an integral role in strategic planning in any wheat breeding programme.

All three rust pathogens are obligate parasites, i.e. they can only functionally grow and reproduce on live green host tissue (Leonard & Szabo, 2005). The three rusts can be distinguished from each other on a wheat plant by means of uredinia. The uredinia are urediniospore-carrying bodies that form when the mycelium growing in susceptible wheat tissue giving rise to dikaryotic spores. These dikaryotic spores are called urediniospores. Urediniospores are released when the uredinia ruptures the epidermis of the wheat plant (Bolton *et al*., 2008). In South Africa, these urediniospores are the three rust pathogens main mechanism for further infection of other nearby susceptible wheat plants (Figure 2.1).

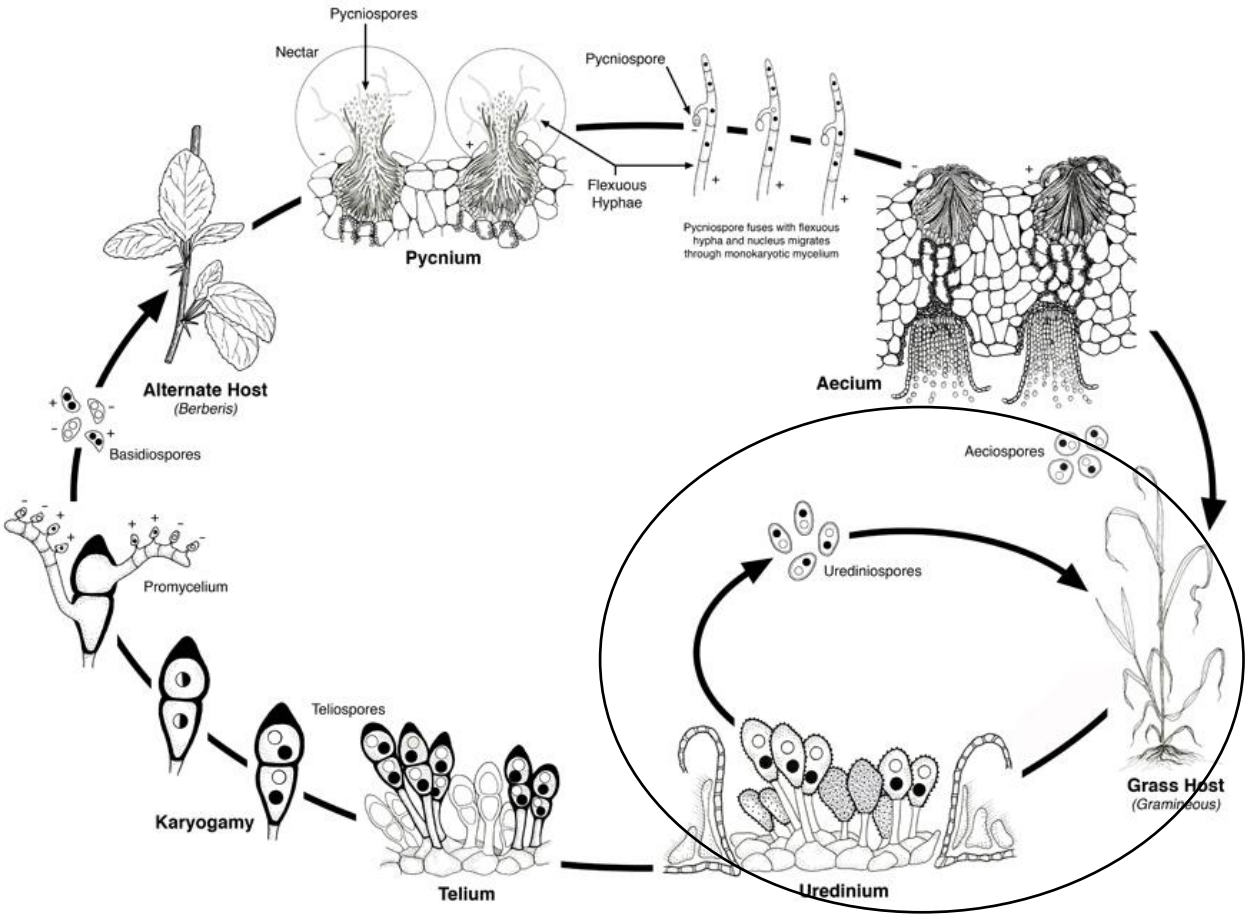


Figure 2.1. Schematic representation of the life cycle of the three rust pathogens. The only phase of importance in South Africa is the circled asexual phase (Leonard & Szabo, 2005).

The rust infection of plant tissue and the development of uredinia cause the epidermis to rupture. The plant experiences a decrease of photosynthetic area due to proliferating uredinia and an increase of transpiration due to ruptured epidermis. The ruptured epidermis also causes a loss in water due to the free movement of water out of the plant through the opening. The combination of these events cause a decrease in the assimilate flow of nutrients and carbohydrates to the ears and ultimately a decrease in the overall yield of wheat (Leonard & Szabo, 2005).

2.2.1. Leaf rust

Leaf rust, caused by *P. triticina*, is the most common and widely distributed of the three wheat rust fungi (Bolton *et al*., 2008). Wheat leaf rust is a foliar pathogen of wheat with the potential to cause extensive loss in grain yield (Boshoff *et al*., 2002). Under epidemic conditions leaf rust causes reductions in yield components including kernel mass, kernels per square meter and grain fill rate, which individually or collectively may result in yield losses of between 7 and 63%, depending on the cultivar's susceptibility to the pathogen (Boshoff *et al*., 2002).

In the 1980's, most of the local wheat cultivars in South Africa were susceptible to leaf rust and localized epidemics occurred frequently in the Western Cape and irrigation areas in other provinces (Pretorius *et al*., 2007). Presently leaf rust is often overlooked as an important disease, internationally and locally, because it does not appear to affect grain yield and quality as much as stem and stripe rust (Leonard & Szabo, 2005; Chen, 2005). Recently leaf rust has started to become quite aggressive in the northern irrigation areas of South Africa. Here leaf rust was not a prominent problem in the years preceding 2008 (F.P. Koekemoer, personal communication, 2011).

Leaf rust thrives when the humidity is high and the daily temperature averages 15 - 22 °C. Under such favourable conditions new spores can be formed every 7 – 10 days (Boshoff *et al*., 2002). Typical symptoms of the disease are round to oval shaped orange-red uredinia which could either be found as groups or as a random spread of pustules on the leaf tissue. The uredinia of leaf rust are up to 1.5mm in diameter, are often surrounded by chlorotic plant tissue and carry huge amounts of spherical urediniospores. These urediniospores group together and are typical 15-30µm in size (Bolton *et al*., 2008). The urediniospores spread, germinate and infect nearby susceptible wheat plants. Due to the colonization of uredinia on the leaf surface and the rupturing of the epidermis, photosynthesis and transpiration is hampered. Ultimately the leaves die off and the reduction of

yield are visualized as a reduction in kernel mass, kernels per square meter and grain fill rate (Boshoff *et al*., 2002).

The main *Poaceae* hosts of leaf rust are wheat, triticale and barley. The alternative hosts for leaf rust, which the pathogen uses to complete it's sexual phase, are species from the *Thalictrum* and *Isopyrum* genus (Bolton *et al*., 2008). In South Africa the sexual phase has not yet been found due to the non existence of alternative hosts (Pretorius *et al*., 2007). The pathogen survives by means of dormant mycelia or urediniospores on volunteer wheat plants in the off season (Bolton *et al*., 2008).

Genetic resistance is the preferred method to reduce losses due to leaf rust. Sixty leaf rust resistance genes have already been designated in wheat (McIntosh *et al*., 2007). Most leaf rust resistance genes confer pathotype specific resistance in a gene for gene manner; however wheat cultivars relying on pathotype specific resistance often lose effectiveness within a few years by imposing selection for virulent leaf rust pathotypes (McIntosh *et al*., 1995). Due to this phenomenon a lot of high yielding cultivars have become susceptible because they only relied on one major gene for resistance. Some of these cultivars are still being cultivated due to their good environmental adaptability and yield, and are being kept rusts-free by fungicide applications (Pretorius *et al*., 2007).

2.2.2. Stem rust

Stem rust caused by *P. graminis* Pers. f. sp. *tritici* is an economically important disease of bread wheat worldwide (Singh *et al*., 2011). In South Africa, the first documented epidemic of stem rust occurred in the south-western parts in 1726 (Pretorius *et al*., 2007). Due to expanding wheat production these epidemics became a recurring phenomenon, and became particularly severe in the winter-rainfall production regions of the Western and Eastern Cape, as well as in the summer-rainfall regions of the Free State (Pretorius *et al*., 2007). The most recent epidemic was the 1984 stem rust epidemic on *Sr24*-derived wheat cultivars in the Albertinia area of the Western (Pretorius *et al*., 2007).

Stem rust is primarily a disease occurring in warm weather areas, but it can cause severe damage to susceptible wheat crops over a broad geographical region. A crop that appears healthy three weeks before harvest can be devastated in a relative short amount of time if sufficient inoculum arrives from a heavily infected wheat crop in a neighbouring region (Singh *et al*., 2011). When stem rust

causes severe infection of the wheat stems, it interrupts nutrient flow to the developing heads, which ultimately results in shrivelled grain. In addition, stems weakened by rust infection are prone to lodging and further cause of yield loss (Roelfs *et al*., 1992).

Stem rust is especially problematic in the winter-rainfall wheat production regions of South Africa. The Mediterranean climate of this area makes stem rust an annual occurrence due to the temperate to warm wet conditions this area encounters during spring when wheat is starting to mature. These conditions are ideal for the development and spread of stem rust that thrives on warm moist conditions (Pretorius *et al*., 2007; Singh *et al*., 2011). During the summer months the inoculum of stem rust do not dissipate due to periodic summer rain that fuel volunteer wheat plants and wild barley (*Hordeum murinum*) on which it can survive (Le Roux & Rijkenberg, 1987).

The oblong brown-red uredinia of stem rust can be found on the stems, both on the upper and lower surface of the leaves and on the glumes (Leonard & Szabo, 2005). The uredinia can measure up to 3mm X 10mm in dimensions, which is rather large in comparison to leaf rust. The urediniospores are ovoid in shape and has dimension of 15-20µm X 40-60 µm (Wiese, 1987). Stem rust develops best under moist and warm conditions. Optimal temperature is at 26°C, and growth is stunted below 15°C and above 40°C (Leonard & Szabo, 2005). Due to this wide temperature spectrum where the pathogen can grow and multiply, stem rust is considered as one of the most important wheat diseases that can affect the majority of the world wheat production areas (Singh *et al*., 2011). The alternative hosts for stem rust, which the pathogen uses to complete it's sexual phase, are the *Barberis* spp. (Leonard & Szabo, 2005). In South Africa the sexual phase cannot take place due to the non-availability of the alternative hosts; however the pathogen uses dormant mycelia or urediniospores to survive the summer months (Pretorius *et al*., 2007).

2.2.3. Stripe rust

Stripe rust is caused by *P. striiformis* Pers. f. sp. *tritici* that infects the green tissues of cereal crops and grasses. Infection can occur anytime, from the one leaf stage to plant maturity, provided plants are still green. As with leaf and stem rust, stripe rust also is found in all parts of the world where wheat is cultivated (Chen, 2005). The disease starts to infect very early and can therefore start to cause damage earlier on in the growing season than leaf rust or stem rust (Chen, 2005). Temperature between 10°C and 15°C along with high humidity is optimal for stripe rust development and spread, although this scenario has also changed a bit from the first research work

that was done on stripe rust (Chen, 2005). Due to new pathotypes that have formed, stripe rust now has a broader temperature range in which it can infect wheat (Chen, 2005).

After it's initial discovery in 1997 in South Africa on an experimental wheat block in Stellenbosch, stripe rust has occurred annually in the cooler and wetter production areas of the Western Cape, eastern Free State and KwaZulu-Natal (Pretorius *et al*., 2007). Like with leaf and stem rust, volunteer or off season wheat plants are big sources for a continuous flow of inoculum to the next wheat planting season. For stripe rust the situation is a bit different for the Free State and KwaZulu-Natal wheat production areas due to the presence of the mountainous enclave country of Lesotho. Due to the production of wheat in Lesotho during the spring and summer months, stripe rust actually has a green bridge where they can grow (Pretorius *et al*., 2007).

Stripe rust infects wheat, barley and a diverse group of other grass species (Boshoff *et al*., 2002). The symptoms are characterized by oblong yellow uredinia (20 µm – 30 µm in diameter) that are arranged along the lateral veins of the leaves. The urediniospores, contained in the uredinia, are spherical in form and 0.5 µm X 1 µm in size (Wiese, 1987). Stripe rust has the ability similar to stem rust to infect almost all the plant tissue of wheat plants (Chen, 2005). The main mechanism of infection is the colonization of uredinia on the leaf surface and the rupturing of the epidermis which hampers photosynthesis and transpiration. Ultimately the leaves die of and the reduction of yield are visualized as a reduction in kernel mass, kernels per square meter and grain fill rate (Boshoff *et al*., 2002).

Until recently it was thought that stripe rust did not have any alternative host on which to complete it's sexual cycle. In a study by Jin *et al* (2010) it was found that the Barberry bush, *Berberis* spp., as in the case of stem rust, is a alternative host for the sexual cycle of stripe rust but due to the absence of this host in South Africa, stripe rust carries on in it's asexual cycle to create new inoculum.

2.2.4. The uredial infection process of the *Puccinia* spp.

After the urediniospores have come into contact with a free film of water on the plant tissue they germinate. Germ tubes of all three rust spores grow along the leaf surface until it reaches one of the stomata. At the stomata the germ tubes of both leaf and stem rust stops elongating and protoplasm flows towards the tip to form an appressorium over the stomatal aperture (Figure 2.2.a).

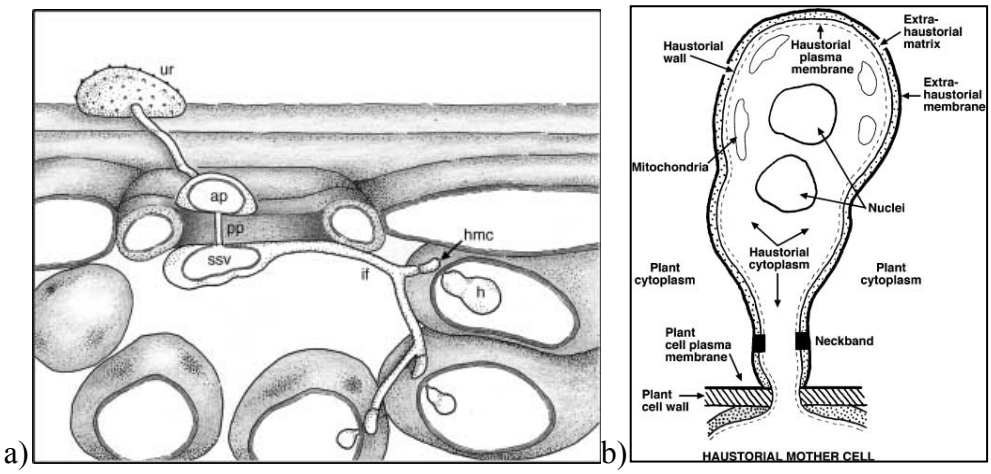


Figure 2.2. a). Schematic representation of the infection structures that is formed during the early stages of uredinial development by *Puccinia* species: ur = urediniospore, ap = appressorium, pp = penetration peg, ssv = substomatal vesicle, if = infection hypha, hmc = haustorial mother cell, h = haustorium. b). Diagram of a haustorium (Leonard & Szabo, 2005).

The formation of the appressoria occurs within 24 hours after inoculation (Bolton *et al*., 2008). The stomata usually close promptly in response to appressorium formation and remains closed in the presence of the appressorium (Bolton *et al*., 2008). A penetration peg originating from the appressorium pushes through the closed stomatal guard cells to gain entry into the intercellular space within the host where the fungus forms an elongated substomatal vesicle (Figure 2.2.a). In contrast to leaf and stem rust, stripe rust does not form an appressorium when it's germ tube has reached the stomata. It's germ tube can gain direct access into the stomata without the formation of such a specialized structure (Wiese, 1987).

From the substomatal vesicle an infection hypha begins to grow inward to the plant cells. When the tip of the infection hypha comes into contact with a host cell, it creates a haustorial mother cell that is separated from the hypha by a septum (Figure 2.2.b.). The haustorial mother cell produces a narrow infection peg that penetrates the host cell wall by means of enzymatic dissolution and pressure (Leonard & Szabo, 2005). When penetrating the cell wall, a specialized fungal hypha expands to form an enlarged haustorium in the periplasmic space of the host cell (Figure 2.2.a & b).

While the haustorium is forming in the periplasmic space, the infection hypha on the outside may produce a new branch just proximal of the first haustorial mother cell. This new branch resumes the growth of the infection hypha and forms a new haustorial mother cell and haustorium when it

comes into contact with another host cell. This process may be repeated, however, by now the nutrient reserves of the original urediniospore have been depleted and further development now depends upon the success of the haustorial in extracting nutrients from the host without inducing a resistance response (Leonard & Szabo, 2005). If the pathogen does not trigger a resistance response, the normal direction of phloem transport of the plant is altered to divert nutrients to the infected tissue at the expense of actively growing plant tissue (Leonard & Szabo, 2005). This change is characterized by massive increases of respiration and accumulation of cytokines in the infected area.

As the wheat plant mature and the initial infection develop, infection mycelium beneath the epidermis of the plant tissue start forming uredinia. These uredinia are fungal bodies that carry dikaryotic urediniospores. As the uredinia develop they set out in size until they break through the epidermis. This can be seen by the naked eye as prominent orange (leaf and stem rust) or yellow (stripe rust) pustules on the leaves, glumes or stems of the wheat plant (Bolton *et al*., 2008; Leonard & Szabo, 2005; Chen, 2005).

When the uredinia ruptures the epidermis and sets the urediniospores free, the asexual phase of the life cycle of the three rust pathogens is complete. This cycle can continue as long as conditions are favorable for the rust fungi to proliferate in this manner (Singh *et al*., 2002).

When the wheat plant is starting to mature and follow its normal ripening phase the rust fungi detects it and start to form structures to survive to the next cycle. These survival structures are seen as teliospores. Teliospores are dikaryotic, brown-black spores with thick, smooth walls that enable it to withstand the harsh conditions during hot summer months (Bolton *et al*., 2008). In areas where their alternative hosts are present, the teliospores allow the rust to infect the alternative host in autumn where they can complete their sexual phase. The alternative hosts for all three rust pathogens are not present in South Africa and due to this they cannot complete their sexual phase. The only life cycle rust follows in South Africa is the asexual phase, where they depend on surviving the summer months by means of asexual urediniospores or dormant pro-mycelium (Boshoff *et al*., 2002).

2.3. Breeding for rust resistance

Cereal rust diseases, in particular wheat rusts, have played an integral role in the history of crop production in South Africa. Through manuscripts it was found that wheat and barley production can be traced back to 1652. These crops were grown by the first Dutch settlers to provide food to passing ships and the growing community of the Cape of Good Hope settlement (Pretorius *et al*., 2007).

The origin of the first rust on South African wheat is unclear, it is suggested that stem rust on wild grasses and rye started parasitizing wheat. Lombard (1987) suggested that in the early 17th century a combination of factors could have established stem rust in South Africa. These factors were introduction of wheat by early Dutch settlers and the establishments of migratory urediniospores through wind dispersal on a new receptive site. With these early wheat cultivations in the Cape Province a receptive area were created where wind dispersed urediniospores, carried by the natural jet stream migratory route, could have established and start to colonize the wheat. It is also suggested that somatic recombination could have given rise to urediniospores that could bridge the gap and make the transition between these wild grasses and rye to infect wheat. A series of studies involving deliberate coinoculation of susceptible hosts with a diverse group of known pathotypes of *Puccinia gaminess* f. sp. *tritici* recorded the appearance of a range of novel stem rust pathotypes (Burdon & Silk, 1997). Lombard (1987) found that the wild grasses *Thinopyrum disticum*, *Agropyrum scabrum* and *Hordeum leporium* were shown to be potential hosts on which somatic recombination of *Puccinia graminis* f. sp. *tritici* might occur. After the initial finding of stem rust on rye it was not very long since the first stem rust epidemic in the Cape province occurred in 1726 (Lombard, 1987).

Since the first introduction of wheat rust, sexual recombination has been prevented due to the absence of all three rust pathogens alternative sexual hosts in South Africa. As a consequence, the pathogen population has developed largely as a series of clonal lineages that have each expanded from founding ancestral isolates by means of asexual reproduction and mutation, to produce a truly wide array of different rust pathotypes (Pretorius *et al*., 2007). These pathotypes have a virulence range that reflects the resistance gene deployment strategies used in wheat in South Africa (Pretorius *et al*., 2007). According to Knott (1989) the evolution of rust populations in the field were often driven by the resistant cultivars produced by the wheat breeders and grown by the farmers. The effect of new rust pathotypes migrating into South Africa is still a big contributor of

wheat cultivars being rendered susceptible when a new virulent pathotype makes it's appearance in the country; as with the case of stripe rust first being reported in 1996 and with the new Ug99 lineage pathotype, PTKST being reported in 2010 (Pretorius *et al*., 1997; Pretorius *et al*., 2010).

2.3.1. Genetic plant resistance

The ability of the plant to recognize and stop or slow down further growth and colonization of any pathogen is attributed to its resistance (Parlevliet, 1993).

Pathogenicity of a plant pathogen is its ability or inability to infect and cause disease on a certain host plant genotype. When looked at the interaction between the host plant and a certain pathotype, pathogenicity can be seen as virulent or avirulent (Parlevliet, 1993). When a pathotype is avirulent for a certain plant genotype, the plant genotype recognizes the pathogen and subsequently initiates a resistance reaction. When a pathotype is virulent for a certain plant genotype, the plant does not recognize the pathotype and it continues to infect and colonize; which in such a case the plant is not resistant to that pathotype (Flor, 1971; Parlevliet, 1993). There can be distinguished between two types of plant resistance namely vertical and horizontal resistance (Van der Plank, 1968; Parlevliet, 1993).

Vertical resistance, also known as pathotype specific resistance, works on a gene-for-gene basis where it is facilitated by an interaction between corresponding gene products of the infecting pathotype and the host plant (Flor, 1971). This type of resistance gives the plant an immediate hypersensitive reaction response against a corresponding avirulent pathotype (Flor, 1971; Parlevliet, 1993). The hypersensitive reaction is visualized as an immediate programmed cell death response where it destroys off its own cells in the surrounding area the pathogen initiated its infection. These sequential cell deaths enclose the infecting area and stop any further pathogen growth. Plant disease resistance is conveyed when the pathotype possesses a avirulence gene (*avr*-gene) which encodes a gene product that is recognized by the plant when it interacts with the encoded host plant's resistance gene (R-gene) product (Flor, 1971). When a pathotype has a virulence gene, there will be no recognition from the plant's side when interaction occurs between the two gene products. This will subsequently lead to a non hypersensitive response (non resistance) reaction and further infection will continue (Flor, 1971). This latter interaction is seen as a compatible relationship between the rust and the plant (Leonard & Szabo, 2005). This gene-for-gene interaction is caused by a major resistance gene which is inherited genetically. These genes are normally dominant in

nature (Parlevliet, 1993). Due to the "boom-and-bust" phenomenon, which happens when a popular cultivar with a very effective major resistance gene is extensively utilized, the life span of major resistance genes is usually short-lived. It is the direct interaction and aggressiveness of these genes that is the cause of their demise due to the profound selection pressure it subject the pathotypes under to overcome it (Singh *et al*., 2005; Parlevliet, 1993).

Horizontal resistance, also known as durable resistance, is characterized as resistance that, despite continues exposure to a pathogen, remains effective for a relative longer time span than major genes (Parlevliet, 1993). This type of resistance can be caused by a single resistance gene with a durable effect or by a number of minor resistance genes which has an accumulating effect (Parlevliet, 1995; Singh *et al*., 2005). The additive effects of these minor genes are associated with resistance reactions that cause slow disease progress under field conditions against a broad-spectrum of pathotypes. Unlike major resistance genes, durable resistance genes are associated with retarding pathogen growth and not by the hyper sensitive response (Parlevliet, 1993; Singh *et al*., 2005). Other than major genes which are triggered when the pathogen invades the intercellular spaces of the plant, durable resistance genes are already encoded before the pathogen infects it (Parlevliet, 1993). Durable resistance genes do not have the aggressiveness of major resistance genes due to their relative smaller effect and their non specificity to pathotypes. Due to this, durable resistance genes need to be incorporated with other resistance genes to insure an effective disease resistance (Singh *et al*., 2005; Singh *et al*., 2011), because of the ability of durable resistance to still give the pathogen a chance to complete its life cycle, the pathotype is never urged to overcome this resistance, like with major gene resistance (Parlevliet, 1993). Cultivars carrying durable resistance genes normally show high infection reactions during the seedling growth stage but reduced infection reactions in the adult plant growth stage (Singh *et al*., 2005).

2.3.2. Origin of new variants of rust pathotypes

The ability of rust to stay one of the most important wheat diseases in the world is being attributed to it's very effective evolutionary capabilities and it's ability to produce immense amounts of spores, that can be wind dispersed for thousands of kilometres. Newly developed or introduced pathotypes of rust to certain countries can be virulent to genes that are predominantly used by these countries. Ultimately this can have a big effect on those countries wheat sustainability and production. The monitoring of wheat rust populations worldwide is very important to allow wheat

breeders and pathologists to anticipate and prepare for the occurrence of new pathotypes that might potentially threaten wheat production (Kolmer, 2005). Wheat rust, as with most organisms, relies primarily on the processes of mutation and recombination as the ultimate source of genetical variation (Burdon & Silk, 1997). Gene flow, caused by migration between different rust populations, supplement these two processes in introducing new sources of variation in situations where sexual reproduction takes place (Burdon & Silk, 1997). Of the three sources of variation in rust pathogen populations, migration is perhaps the simplest, although it's contribution to diversity may be underestimated (Burdon & Silk, 1997).

2.3.2.1. Mutation

How mutation contributes to diversity of a pathogen populations depends largely on the pathogen's inherent rate of mutation, its ploidy level (being either haploid, diploid or dikaryotic), the size of the pathogen population and the selective advantage bestowed upon the mutant phenotype (Burdon, 1992). Genes for virulence in the rust pathogens, which is dikaryotic, are generally recessive and mutations on these genes occur much more frequently than on dominant genes (Flor, 1956; Knott, 1989). The mutation rate for a single gene is in the order of 1 X 10^{-5} or 10^{-6} (Knott, 1989; Falconer & Mackay, 1996). For a mutation of a recessive gene in a dikariote to take full effect, a mutation needs to take place on both loci of the recessive gene. This entails that the frequency of a single spore having a double mutation is in the order of 1 X 10^{-10} or 10^{-12} (Knott, 1989). It is estimated that half a hectare of wheat with a stem rust severity of 10% can produce a trillion spores ($1X10^{12}$) (Rowell & Roelfs, 1971). This can mean that if a field of a new cultivar carrying a single gene for resistance is surrounded by fields of susceptible cultivars, large numbers of spores will be blown into it and a few will be virulent mutants. If mutants can infect the plants and sporulate, the extreme selection pressure will result in a rapid increase of this new mutant (Knott, 1989). Spontaneous mutation is a powerful contributor to new variation in many pathogen populations and is the origin of the majority of new pathotypes of *Puccinia graminis* f. sp. *tritici* that has arisen in the various clonal lineages in Australia, the United States of America and South Africa (Burdon & Silk, 1997; Pretorius *et al*., 2007).

2.3.2.2. Recombination

Recombination in plant pathogens occur either through sexual reproduction or through a process of somatic hybridization. Both these mechanisms have considerable importance in genotypic diversity in a rust population (Burdon & Silk, 1997).

In areas where the alternative hosts of these rust pathogens can be found sexual reproduction occurs annually. This type of reproduction can lead to the generation of an enormous number of incipient clonal lineages as was evident during the 1970's, the time that eradication of Barberry was not that actively practised in the United States. Before the extensive eradication of Barberry, more than seventeen pathotypes were detected on a yearly basis. Since that time, the numbers has noticeably fallen to only six or seven pathotypes that are now commonly found on a routinely basis (Burdon & Silk, 1997).

Somatic hybridization involves the exchange of nuclei between fungal hyphae between pathotypes of the same specie infecting the same host (Knott, 1989). The rust fungi are a dikaryotic basidiomycete. In these fungi two genetically different nuclei normally exist together in a stable state. This nuclear ability of the basidiomycetes can help to generate a maximum of two new combinations of characters (Burdon & Silk, 1997). Further variation may be generated when nuclear exchange, by means of somatic hybridization, is followed by nuclear fusion, mitotic recombination and subsequent non meiotic rearrangement of chromosomes (Burdon & Silk, 1997). Somatic hybridization could have been the driving factor behind the transition of rye infecting stem rust to wheat infecting stem rust in the early phases of wheat production in South Africa in the 17th century (Lombard, 1987).

2.3.2.3. Migration

The rust's urediniospores have the inherent ability to be carried by upper air jet streams for thousands of kilometres from initial infection sites (Kolmer, 2005). This ability is attributed to the physical form of these spores. Urediniospores of all three rusts have protrusions on their outside that increases their surface area so that they can be carried more easily by the jet streams. Due to the sub zero temperatures and low moisture content where these jet streams blow, the spores can stay viable for long periods of time (Knott, 1989). Periodic downward air streams bring these spores into contact with weather systems which deposit's the spores onto the wheat hosts by means of rain

(Knott, 1989). Examples of such migratory patterns of wheat rusts are the migration of a very virulent stripe rust pathotype from Kenya to India and the migration of the stem rust pathotype Ug99 from Uganda to Yemen (Hodson *et al*., 2005; Singh *et al*., 2011).

2.3.3. Leaf rust

The first leaf rust work in South Africa was conducted in the mid 1930's when Verwoerd (1937) surveyed the different leaf rust pathotypes occurring in South African wheat fields. During these surveys five different pathotypes were identified. After this initial survey work done on leaf rust, it stopped until a renewed effort were started up again during the period from 1983-1988 (Pretorius *et al*., 1987; Pretorius & Le Roux 1988; Pretorius *et al*., 1990). The renewed effort was attributed to regular epidemics occurring in the Western Cape and irrigation areas due to susceptibility of most of the predominant wheat cultivars grown (Pretorius *et al*., 1987). During these extensive surveys, sixteen different pathotypes have been identified (Pretorius *et al*., 2007). It was found that variation between the pathotypes occurs mostly at the *Lr10*, *Lr14a*, *Lr17*, *Lr24* and *Lr26* loci (Pretorius *et al*., 2007). Tarefe *et al*. (2011) isolated a new leaf rust pathotype in 2009 from a rust trap nursery in the Western Cape. This pathotype, designated as 3SA145, showed combined virulence for *Lr12*, *Lr13* and *Lr37* (Tarefe *et al*., 2011). Although virulence to *Lr12* and *Lr13* has been known in different leaf rust pathotypes in South Africa, this is the first report of combined virulence for these genes (Tarefe *et al*., 2011). Because of a very aggressive fungicide application on commercial wheat fields in the Western Cape, and the cultivation of cultivars with effective resistance, leaf rust has become less prevalent in recent years (Pretorius *et al*., 2007). The predominant leaf rust pathotypes in South Africa spreading the period from 2009 – 2011 were the newly identified 3SA145 pathotype and the older 3SA133 pathotype (Dr. T. Tarefe, Small Grain Institute, Bethlehem, South Africa, personal communication, 2012). Information regarding leaf rust genes being deployed in South Africa is listed in Table 2.1.

2.3.4. Stem rust

In South Africa stem rust pathotyping began in 1920 and continued up until 1973 (Verwoerd, 1937; De Jager, 1980). In 1960, interest in stem rust grew that led to the implementation of an improved differential sets and more regular surveys. The improved differential sets showed that well

established stem rust epidemics were in fact caused by separate pathotypes. During these surveys eighteen different stem rust pathotypes were identified on host plants such as *Triticum aestivum*, *Agropyron distichum*, *Hordeum murinum*, *H. vulgare*, *Lolium italicum*, *Bromus maximus* and *Dactylis glomerata* from 1920 to 1973 (Pretorius *et al*., 2007). Since 1980 stem rust resistance became essential for all new wheat cultivars released in South Africa. The Agricultural Research Council (ARC), in order to help in facilitating wheat breeding companies in breeding resistant cultivars, started to conduct annual stem rust pathotype surveys (Pretorius *et al*., 2007).

Stem rust has acquired virulence to many resistance genes, including virulence to resistance genes prevalent in triticale (Smith & Le Roux, 1992). The acquiring of virulence by stem rust pathotypes were largely attributed to the widespread use of a single cultivars that has created immense selection pressure that fuelled the rapid evolution of virulent pathotypes (Pretorius *et al*., 2007). With the introduction of new resistant wheat cultivars the stem rust landscape also evolved in that manner. In 2007 the most prevalent stem rust pathotype in South Africa is UVPgt55 (2SA88) which is virulent for the majority of the most major resistance genes in South Africa (Pretorius *et al*., 2007).

The stem rust resistance gene *Sr31* has been used in agriculture on the largest scale since the 1980s in the well known Veery cultivars from CIMMYT (McIntosh *et al*., 1995). Veery#3 was used as direct released cultivar in many major wheat producing countries due to it's adaptability and yield potential (McIntosh *et al*., 1995). These cultivars carried the rye translocation 1BL.1RS which was associated with increased grain yields and resistance to all three rusts and powdery mildew as it carried resistance genes for all these diseases on the same translocation (Singh *et al*., 2011). Large-scale deployment of *Sr31* surprisingly did not result in it's breakdown until the detection of pathotype Ug99 in Uganda in 1999 (Pretorius *et al*., 2000). This pathotype were subsequently also found in Kenya and eventually made it's way to Ethiopia in 2004 (Singh *et al*., 2006). Due to the predominant use of *Sr31* in the majority of the major wheat producing countries, global wheat production is at risk (Singh *et al*., 2011). The Global Rust Initiative (www.globalrust.org) has been launched in response and includes an emergency crossing programme to deploy effective resistance genes accompanied by a massive testing of advanced lines in the affected areas (Jin & Singh, 2006). The South African pathotype, UVPgt55 (2SA88), was compared to Ug99 using molecular markers and it was found to resemble Ug99 closely (Visser *et al*., 2009). UVPgt55 avirulence/virulence composition is also identical to Ug99 except that it lacks virulence for *Sr24* and *Sr31* (Pretorius *et*

al., 2007). Initially Ug99 was not a threat in South Africa as cultivars with *Sr31* are not common, due to the presence of the linked secalin gene which codes for sticky dough (Pretorius, *et al*., 2007). In November 2009 a stem rust isolate, PTKST, were found on the *Sr31* carrying line Federation4*/Kavkaz in South Africa. This line showed a susceptible response to stem rust in a disease nursery near Greytown in KwaZulu-Natal, South Africa (Pretorius, *et al*., 2010). Simple-sequence repeat (SSR) analysis with selected primer pairs showed that PTKST clusters with isolates belonging to the Ug99 lineage (Visser *et al*., 2009). It was also found that PTKST has subsequently acquired virulence for *Sr24*, making it a substantial threat for South Africa (Pretorius, *et al*., 2010). An Indian rust pathotype, PKTSC, has recently become virulent to *Sr25* (Jain *et al*., 2009), which was one of the few remaining effective major resistance genes against Ug99. If Ug99 acquires *Sr25* virulence as well, it will combine virulence to most of the major rust resistance genes in use globally. Information regarding stem rust genes being deployed in South Africa is listed in Table 2.2.

2.3.5. Stripe rust

In comparison with the other two rust pathogens, stripe rust has a shorter history in South Africa. Stripe rust was first recorded in South Africa in 1996 (Pretorius, *et al*., 1997). The first stripe rust pathotype found in South Africa was 6E16A-, a common Middle Eastern pathotype. With the addition of *Yr25* virulence 6E16A- has become 6E22A-. Stripe rust commonly occurs in the Western Cape, KwaZulu-Natal and eastern Free State which are cooler and wetter. A third pathotype, 7E22A-, which has virulence to *Yr1,* has been found in Lesotho. This new pathotype should not threaten South African wheat as no local cultivars have *Yr1* (Pretorius *et al*., 2007).

Stripe rust has been costly to the industry, as farmers now have to cope with an additional threat and many commercial cultivars are not stripe rust resistant. Wheat breeding programmes have also been harmed, loosing up to 60% of early generation breeding material (Boshoff *et al*., 2002). Most of the stripe rust resistant cultivars available in South Africa are not resistant to stem rust and this has lead to an increase in stem rust (Pretorius *et al*., 2007). Information regarding stripe rust genes being deployed in South Africa is listed in Table 2.3.

Table 2.1. Efficiency of leaf rust genes in South Africa (Pretorius *et al*., 2007; Tarefe *et al*., 2011; Le Maitre, 2010)

Gene	Avirulent rust pathotypes	Virulent rust pathotypes	Origin	Chromosomal location	Reference
Lr10	UVPrt3 (3SA123), UVPrt5 & UVPrt19	UVPrt2, UVPrt4, UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt10 (3SA126), UVPrt13 (3SA140) & 3SA145	*T. aestivum*	Short arm of chromosome 1A	Dyck & Kerber, 1971, McIntosh *et al*., 1995
Lr14a	UVPrt3 (3SA123) & UVPrt5	UVPrt2, UVPrt4, UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt10 (3SA126), UVPrt13 (3SA140), UVPrt19 & 3SA145	*T. turgidum* var. *diccocum* cv. Yaroslav	Long arm of chromosome 7B	Law & Johnson, 1967; McIntosh *et al*., 1995
Lr17	UVPrt2, UVPrt3 (3SA123), UVPrt4, UVPrt5, UVPrt9 (3SA133),	UVPrt8 (3SA132), UVPrt10 (3SA126), UVPrt13 (3SA140), UVPrt19 & 3SA145	*T. aestivum*	Short arm of chromosome 2A	Dyck & Kerber, 1977; Bariana & McIntosh, 1993; McIntosh *et al*., 1995
Lr19	Provides sufficient resistance to all known pathotypes in South Africa		*Thinopyrum ponticum*	Long arm of chromosome 7D	Sharma & Knott, 1966; McIntosh *et al*., 1976; McIntosh *et al*., 1995
Lr24	UVPrt2, UVPrt4, UVPrt5, UVPrt10 (3SA126) & 3SA145	UVPrt3 (3SA123), UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt13 (3SA140) & UVPrt19	*Th. ponticum*	Long arm of chromosome 3D	Smith *et al*., 1968; McIntosh *et al*., 1995
Lr26	UVPrt2, UVPrt3 (3SA123), UVPrt4, UVPrt5, UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt10 (3SA126) & UVPrt19	UVPrt13 (3SA140) & 3SA145	*Secale cereale* cv. Petkus	Long arm of chromosome 1B (1BL.1RS)	Zeller, 1973; Lukaszewski, 2000; McIntosh *et al*., 1995
Lr34	Provides sufficient adult plant resistance to all known pathotypes in South Africa		*T. aestivum*	Short arm of chromosome 7 D	Singh, 1992; McIntosh *et al*., 1995
Lr37	UVPrt2, UVPrt3 (3SA123), UVPrt4, UVPrt5, UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt10 (3SA126), UVPrt19 & UVPrt13 (3SA140)	3SA145	*T. ventricosum*	Short arm of chromosome 2A	Bariana & McIntosh, 1993; McIntosh *et al*., 1995

Table 2.2. Efficiency of stem rust genes in South Africa (Pretorius *et al*., 2007; Pretorius *et al*., 2010; Le Maitre, 2010)

Gene	Avirulent rust pathotypes	Virulent rust pathotypes	Origin	Chromosomal location	Reference
Sr2	Provides adult plant resistance to all the known pathotypes in South Africa		*T. turgidum* var. *diccocum* cv. Yaroslav	Short arm of chromosome 3B	McFadden, 1930; Knott 1968; McIntosh *et al*., 1995
Sr24	UVPgt50 (2SA4), UVPgt51 (2SA36), UVPgt53 (2SA102), UVPgt54 (2SA55), UVPgt55 (2SA88), UVPgt56 (2SA102K) & UVPgt57 (2SA105)	UVPgt52 (2SA100) & PTKST	*Th. ponticum*	Long arm of chromosome 3D	Smith *et al*., 1968; McIntosh *et al*., 1995
Sr25	UVPgt50 (2SA4), UVPgt51 (2SA36), UVPgt52 (2SA100), UVPgt53 (2SA102), UVPgt54 (2SA55), UVPgt55 (2SA88) & PTKST		*Th. ponticum*	Long arm of chromosome 7D	Sharma & Knott, 1966; McIntosh *et al*., 1976; McIntosh *et al*., 1995
Sr31	UVPgt50 (2SA4), UVPgt51 (2SA36), UVPgt52 (2SA100), UVPgt53 (2SA102), UVPgt54 (2SA55), UVPgt55 (2SA88), UVPgt56 (2SA102K) & UVPgt57 (2SA105)	PTKST	*Secale cereale* cv. Petkus	Long arm of chromosome 1B (1BL.1RS)	Zeller, 1973; Lukaszewski, 2000; McIntosh *et al*., 1995
Sr38	UVPgt50 (2SA4), UVPgt51 (2SA36), UVPgt52 (2SA100), UVPgt53 (2SA102), UVPgt54 (2SA55), UVPgt56 (2SA102K) & UVPgt57 (2SA105)	UVPgt55 (2SA88) & PTKST	*T. ventricosum*	Short arm of chromosome 2A	Bariana & McIntosh, 1993; McIntosh *et al*., 1995

Table 2.3. Efficiency of stripe rust genes in South Africa (Pretorius *et al*., 2007; Pretorius *et al*., 2010; Le Maitre, 2010)

Gene	Avirulent rust pathotypes	Virulent rust pathotypes	Origin	Chromosomal location	Reference
Yr1	6E16A- & 6E22A-	7E22A-	*T. aestivum*	Long arm of chromosome 2A	Lupton & Macer, 1962; Bariana & McIntosh, 1993; McIntosh *et al*., 1995
Yr9	6E16A-, 6E22A- & 7E22A-		*Secale cereale* cv. Petkus	Long arm of chromosome 1B (1BL.1RS)	Zeller, 1973; Lukaszewski, 2000; McIntosh *et al*., 1995
Yr17		6E16A-, 6E22A- & 7E22A-	*T. ventricosum*	Short arm of chromosome 2A	Bariana & McIntosh, 1993; McIntosh *et al*., 1995
Yr18	Provides adult plant resistance to all the known pathotypes	in South Africa	*T. aestivum*	Short arm of chromosome 7D	Singh, 1992; McIntosh *et al*., 1995
Yr25	6E16A-	6E22A- & 7E22A-	*T. aestivum*	Chromosome 1D	Calonnec & Johnson, 1998; McIntosh *et al*., 1995

2.4. Resistance breeding strategies

The genetic improvement of self-pollinating crops, such as wheat, comprises of three main phases. The first phase being the introduction and recombination of genes for enlarging genetic variation or incorporating sought after traits by means of planned crosses. The second phase is the identification and selection of newly developed lines from the crosses and developing inbred/homozygous lines from them (Koebner & Summers, 2003). The third and last phase is the extensive evaluation of the pure breeding lines over multi localities to identify their adaptability and stability over a wide production area (Marais & Botes, 2009; Baenzinger & Peterson, 1992; Inagaki *et al*., 1998). The most important phase of a breeding programme can be seen as the selection of the crossing parents. The parents are chosen in such a way that they complement each other for certain traits, e.g. one parent might be high yielding but susceptible to a major rust pathotype whereas the other might not yield as good but have excellent rust resistance. When combining these two genotypes, high yielding resistant genotypes can be selected out of the progeny. After the initial cross between the two parents, single plant selection is practised on the early generation segregating progeny (Koebner & Summers, 2003). New variation may be introduced, normally through a process of pre-breeding that limits co-transfer of undesired chromatin and preserves earlier selection gain (Marais & Botes, 2009). In conventional breeding of self-pollinating crop, genes are fixed through inbreeding after the first cross between the two parents is initiated. In self-pollinators after each successive inbreeding generation, heterozygosity is halved (Marais & Botes, 2009). The size and composition of the plant breeding population is an important consideration for a breeding programme. The larger the number of genes segregating in a population, the larger the population size is required in order to identify specific gene combinations (Marais & Botes, 2009).

2.4.1. Pedigree breeding method

The pedigree breeding method is, in contrast to the newer breeding methods, the conventional breeding method to accumulate genetic recombination in self-pollinating crops such as wheat. The pedigree breeding scheme derives it's name from it's ability to trace the ancestry of each selection made through all subsequent generations to it's origin, which is a specific hybrid plant (Koebner & Summers, 2003). This method was developed in the late 1800's in Europe and Australia (Lupton, 1987), but was first formally described in 1927 (Love, 1927). The pedigree breeding method is based on the foundation where plants are selected for high heritable traits, such as major gene

disease resistance, in the early or segregating generations; and low heritable traits, such as grain quality that is influenced by numerous genes, in a later generation when most of the gene has been fixed and do not segregate anymore.

In it's simplest form, two parental genotypes are chosen to be crossed with each other. After the initial cross between the two parents, single plant selection is practised on the early generation segregating progeny (Bingham & Lupton, 1987). In the first segregating generation (F_2), each plant is genetically unique and segregation takes place at every gene for which the parents differed in their allelic state. Early generation material is usually planted in diseases nurseries where they are artificially inoculated with a predominant disease. Selection in this early generation is performed on individual plants where plants are scrutinized on high heritable traits such as major gene disease resistance and plant hight, influenced by the *Rht* genes. As selection is made by selecting individual plants, progeny of the selected plants is again planted out for the next generation. To capture a good representation of the total variation possible in the F_2, a large amount of progeny from each crossing combination is planted out and visually evaluated and selected (Bingham & Lupton, 1987; Inagaki *et al*., 1998). Selection made from generation to generation causes the selected lines to get more homozygous due to inbreeding and the ratio of segregating individuals decrease with each successive generation. As the lines get more homozygous, selection gets more directed to individual lines rather than the single plants. At the F_5 generation and onwards, selection is made more for quantitative traits, characteristics influenced by more than one gene and which is only able to be evaluated with some level of replication (Bingham & Lupton, 1987). Particularly these quantitative traits include baking quality characteristics and grain yield. When enough seed of each individual line is available, multi-location testing are conducted. The multi-location testing is done to evaluate the selected lines' adaptability over a wide geographic area and their stability over a number of years. This is necessary due to the important role adaptability and stability plays in releasing a new cultivar (Koebner & Summers, 2003). The multi-location tests also allow for testing quality trait expression over diverse environments and identify stabile quality expression. Based on good adaptation of yield and quality characteristics, expressed over a couple of years, final selection is conducted (Bingham & Lupton, 1987).

The pedigree breeding method is still being used by international (Ortiz *et al*., 2007) as well as local breeding programmes. Since the first description of the pedigree breeding system in 1927, new technology that was developed, such as marker-assisted selection (MAS), facilitated this method to make more concurrent selections on traits not that easily evaluated or measured by the breeder's eye

(Ortiz *et al*., 2007; Collard & Mackil, 2008). Ways of accelerating this process emerged in the form of off-season nurseries which allowed breeders to acquire two subsequent generations in a single year (Ortiz *et al*., 2007). This method, which sometimes uses two diverse environments to acquire two subsequent generations in a year, allowed for the development of the high yielding daylight insensitive wheat cultivars which initiated the Green Revolution (Trethowan *et al*., 2007).

2.4.2. Single seed descent (SSD) breeding method

The single seed descent (SSD) breeding method is based on the facilitating rapid growth of wheat plants by decreasing generation time (Knott, 1989; Inagaki *et al*., 1998).

The SSD procedure normally starts at the F_2 generation where one to two seeds are taken from each of the F_2 individual plants obtained from a crossing population. The process is repeated for about three to five generations (F_5 or F_6), or until desired levels of homozygosity are reached (Knott, 1989). From generation to generation the level of homozygosity increases due to fixating heterozygote loci with each generation. By the sixth generation (F_6) of SSD, genotypes are on average 97% homozygous (Knott, 1989).

The main objective of SSD is to produce homozygous lines in the shortest time possible using a single seed per line from each successive generation. To acquire this, SSD plants are grown under artificial conditions that are very conducive for rapid plant growth that shortens generation times. Rapid plant growth can be facilitated under artificial lights, higher plant densities and increased nitrogen fertilization (Knott, 1989). The advantages of this method compared to the pedigree method is that the time to develop a homozygous breeding population is significantly reduced which means that the material can be planted in a yield evaluation trial much quicker (Inagaki *et al*., 1998, Marais & Botes, 2009). When compared to the pedigree method the expected duration since a cross is made to the time a cultivar is released can be reduced by 4-5 years (Marais & Botes, 2009).

In spring wheat breeding programmes, the SSD breeding method is a very effective method to speed up the production of homozygous lines and simultaneously preserving the high level of genetic variability from the initial heterozygous crossing population (Knott, 1989; Vencovsky & Crossa, 2003). The high level of variability of advanced lines is due to the absence of selection practiced during early generations, each individual of a crossing population is maintained from generation to generation (Vencovsky & Crossa, 2003). When comparing the SSD breeding method

to random line selection methods, such as the random bulk system, SSD maintains genetic drift at a lower population level and offers a much better protection against random loss of alleles during each successive generation (Vencovsky & Crossa, 2003). What this entails is that SSD produces better representation of the original population in advanced generations.

2.4.3. Recurrent mass selection (RMS) method

This method is a very popular method in increasing the frequency of advantageous genes (Marais & Botes, 2009). Basically it involves selecting parents, making all possible crosses between them, growing the progeny, selecting for desired characteristics in the progeny, and then intercrossing the selected progeny to start another cycle (Knott, 1989). By doing this genes selected for are enriched into the recurrent breeding material. RMS has been shown to be a very powerful breeding method in the improvement in cross-pollinating crops (Allard, 1999). This method was developed mainly for improvement of polygenic/quantitative traits, which is controlled by multiple genes with small but accumulating effects (Falconer & Mackay, 1996). The underlying objective of this breeding method is to increase the frequency of advantages genes in a breeding population. By doing this the chance is increased of selecting superior genotypes with acceptable polygenic inherited traits (Marais & Botes, 2009). The level of heterozygosity in the breeding population together with the large amount of crossing combinations that can be made, allow for a more complete exploration of polygenic recombination potential (Marais & Botes, 2009).

Due to the ability of self-pollinating crops to rapidly fix genes the opportunity of genetic recombination is strongly reduced (Marais & Botes, 2009). Taking this into consideration when trying to incorporate polygenic traits into a wheat breeding population, the potential of polygenic recombination would not be adequately explored by using only a single cycle of crossing and selection (Marais & Botes, 2009).

For this reason the principles of RMS are equally just as relevant to self-pollinating crops as with cross-pollinating crops. Due to the success shown by RMS of breeding for polygenetic traits in cross-pollinating crops, breeders and researchers looked at ways to incorporate this method of breeding to wheat and other self-pollinating crops (Marais & Botes, 2009). The only major limiting factor this method has in self-pollinating crops is their inherent inability to cross-pollinate on a large scale.

One way of incorporating RMS in wheat is by using male sterility to create an open pollinating wheat plant (Huang & Deng, 1988; Cox *et al*., 1991; Marais *et al*., 2000). The use of the dominant male sterility gene *Ms3* to facilitate mass crossings in a pedigree/recurrent mass selection breeding scheme were already successfully implemented to breed for multi-genic pest resistant wheat lines (Marais & Botes, 2003). Combining an *Ms3* wheat population with the technique of hydroponic tiller culture, little effort and space are needed to facilitate mass random crossings between large numbers of selected male and female wheat parents (Marais *et al*., 2001). The male parents, *viz.* male fertile, who are used in such a scheme is homozygous recessive for the dominant male sterility gene (*ms3ms3*) while the female parent, *viz.* male sterile, is heterozygous for the dominant male sterile gene (*Ms3ms3*). When allowing these materials to intercross, a progeny population is created that segregates in a 1:1 ratio for male sterility and male fertility (Marais *et al*., 2001). After the mass crossings all the progeny can be inoculated at an early stage by predominant rust pathotypes so that selection can be performed during an early plant development stage which allows for only the resistant progeny being kept in the recurrent cycle (Marais *et al*., 2001). By using this method, genes for seedling rust resistance can be enriched in the recurrent mass selection population (Marais *et al*., 2001). The first requirement of a recurrent mass selection programme is to establish a diverse base population with a diverse genetic base for different characteristics such as disease resistance, yield components, adaptability and quality. This is necessary due to the recirculation of male progeny from the initial mass hybridization (Marais *et al*., 2001). This recirculation creates a situation where only the genetic variability of the base population is recycled with each successive generation (Marais & Botes, 2009).

2.5. Biotechnology aided breeding

Most breeding methods are extremely time consuming, as discussed previously with the pedigree and SSD breeding methods. For this reason molecular tools can be applied to reduce the time spent developing new cultivars and to streamline the breeding process. Two of the biotechnology tools often used are the rapid development of homozygote breeding lines through the doubled haploid (DH) method; and the early and effective screening of traits through MAS.

2.5.1. Creating inbred lines by the doubled haploid (DH) method

Presently a number of DH production techniques for wheat are available (Niroula & Bimb, 2009). Each technique has a different mechanism on which it is based such as the androgenesis technique which uses anther/microspore culture, the gynogenesis technique which uses ovary/ovule culture, chromosome elimination following wide hybridization, haploid inducer genes and chemicals (Niroula & Bimb, 2009). In wheat a number of these mechanisms were tested and showed variable success. Regeneration of anther/microspore-derived plants was successful in wheat, as well as, all other main cereal crops (Liu *et al*., 2002). Haploid regeneration from cultured ovaries/ovules has been reported in wheat (Baenzinger *et al*., 2001). However the efficiency of haploid regeneration by this technique is not as high as that in the anther/microspore-culture (Niroula & Bimb, 2009). Despite the limited success, the efficiency of haploid production in wheat through anther/microspore and ovaries/ovules techniques are highly genotype dependent which limit's the use of these two techniques in practical wheat breeding programmes (Niroula & Bimb, 2009).

Presently the DH technique that is mostly employed in wheat breeding programmes are the wide hybridization technique (Niroula & Bimb, 2009). DH development of wheat through wide hybridization were shown to be feasible through two processes namely the *bulbosum* technique and the wheat X maize technique (Barclay, 1975; Riera-Lizarazu & Mujeeb-Kazi, 1990). The *bulbosum* technique was originally developed for the production of DH barley (*Hordeum vulgare*) lines. In a later stage this technique was extended to produce DH wheat lines as well (Barclay, 1975). This technique involves the crossing between wheat and *Hordeum bulbosum*. Wheat facilitates as the female parent and *Hordeum bulbosum* as the male parent. After fertilization seed develops for about 10-14 days after which the embryo is rescued and placed on a growing medium for further growth under *in vitro* conditions. During the development of the hybrid zygote the *bulbosum* chromosomes gets eliminated and a haploid wheat embryo is subsequently developed (Barclay, 1975). As with the anther/microspore and ovaries/ovules techniques, the wheat X *Hordeum bulbosum* are also highly genotype dependent. This is due to the presence of the homozygote recessive wheat crossability genes, *Kr1* and *Kr2*, located respectively on chromosome 5A and 5B. The presence of these genes markedly reduces the crossability between wheat and *Hordeum bulbosum* (Barclay, 1975).

The wheat X maize technique currently is the DH technique most extensively being utilized for haploid production in wheat worldwide (Niroula & Bimb, 2009). Since the first report of embryos being formed when hexaploid wheat is crossed with maize pollen, the maize mediated haploid

breeding technique for wheat has been extensively exploited (Zenkteler & Nitzsche, 1984; Laurie & Bennett, 1986; Laurie & Bennett, 1989; Niroula & Bimb, 2009). It has immense practical application when compared to the andro- and gynogenesis techniques and the *bulbosum* technique. This is because the haploid production via the wheat X maize technique is very simple and does not have the high genotype dependent interaction shown by the other techniques (Niroula & Bimb, 2009).

The wheat X maize haploid process starts when maize pollen is used to fertilize an emasculated wheat floret. The maize pollen germinates and grows into the wheat embryo sac where the wheat egg is fertilized by the maize nuclei. A hybrid zygote with 21 wheat chromosomes and 10 maize chromosomes is produced (Laurie & Bennett, 1989). The hybrid zygotes are karotypically unstable; for this reason the maize chromosomes fail to move to the spindle poles during cell divisions. This phenomenon is possibly due to the inability of the maize chromosome centromeres to attach to the spindle microtubules. Due to this the maize chromosomes are rapidly eliminated after a few cell divisions, which causes a haploid embryo with 21 wheat chromosomes to form (Laurie & Bennett, 1989). In order to initiate seed development the floret with the haploid embryo needs to be treated with a growth hormone such as 2.4-D a day after the wheat X maize cross (Niroula & Bimb, 2009). Embryos are rescued from the seeds 15-21 days after the initial wheat X maize cross and grown *in vitro* on growing mediums. Haploid plants later are treated with colchicine to induce the doubling of the chromosome number and subsequently develop the doubled haploid wheat plant (Niroula & Bimb, 2009).

2.5.2. Marker-assisted breeding

The fundamental premise of plant breeding is the selection of a single plant out of a population of plants that has the specific desired trait being sought. The goal of plant breeding is to bring together more desirable combinations of genes in new cultivars (Collard & Mackill, 2008). In the commonly used pedigree breeding method, selecting for traits with high heritability starts in the early generations. However for traits that is governed by a collection of genes which all together has a accumulative effect (quantitative or low heritable traits), selection is often delayed until the lines become more homozygous in later generations (F_5 or F_6) (Bingham & Lupton, 1987). In these breeding programmes, selection is predominantly done on physical characteristics of the plant. In some instances physical characteristics may also be co-inherited with certain genes of interest. In

such cases physical characteristics associated with these genes are called physical or phenotypic markers. There are several types of phenotypic markers used by plant breeders that is linked to specific traits, for example, pseudo black chaff (PBC) which is linked to *Sr2* stem rust resistance (Brown, 1993), leaf tip necrosis which is linked to the slow rusting leaf rust and stripe rust resistance genes *Lr34*/*Yr18* (Spielmeyer *et al*., 2005) and the red glume colour which is phenotypically linked to the stripe rust resistance gene*Yr10* (Metzger & Silbaugh, 1970). The onset of DNA markers has somewhat overcome the limitations of phenotypic selections by presenting a phenotypic neutral way of selecting for traits (Koebner & Summers, 2003).

Conventional breeding techniques have shown that yield advances in important grain crops are evident over the long term (Trethowan *et al*., 2007). But despite obvious optimism about these continued yield improvements; new technologies are needed to maximize the probability of continoues crop improvement successes (Huang *et al*., 2002). One such technology is DNA marker technology, derived from research in molecular genetics and genomics (Collard & Mackill, 2008). This technology presents great promise for plant breeding in the form of indirect selection capabilities for certain economical important traits. Due to genetic linkage, DNA markers can be used to detect the presence of allelic variation in the genes expressing these traits (Collard & Mackill, 2008). The implementation of DNA markers could greatly increase the efficiency as well as the precision of any plant breeding programme.

Markers may be linked, i.e. has a probability of being co-inherited with the genes influencing the trait. They can also be diagnostic, also known as perfect, if the marker is directly associated with the gene that influences the trait (Gale, 2005).

There are five main factors to take into account when DNA markers are implemented in MAS. These factors are reliability; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism; and cost (Collard & Mackill, 2008).

One of the most important factors in considering any molecular marker is its reliability. This is visualized by the marker's recognition site which needs to be in close proximity to the target gene. A marker must be preferably perfect or less than 5cM genetic distance away from the target gene. MAS reliability can greatly be increased when a perfect or flanking marker can be used. Reliability of the marker is increased if it is inherited in a co-dominant manner. This gives the marker the ability to discriminate between homozygous and heterozygous individual.

DNA quantity and quality used plays an integral role when it comes to considering the implementation of certain molecular marker techniques. Some marker procedures require big amounts of high-quality DNA, which is sometimes a tall order to obtain in practice, and which also can increase the total running cost of the procedure.

The technical procedure of implementing a certain MAS technique is of importance due to the level of simplicity and the time required for the technique. A high-throughput simple and quick method is highly desirable.

The level of polymorphism plays a big role when it comes to choosing a marker. A marker needs to be highly polymorphic in diverse breeding backgrounds that will be able to discriminate between the marker donor and a wide array of cultivated genotypes.

In breeding programmes costis the most important factor when it comes to selecting different techniques to be used in selection, MAS is not an exception. The marker assay must be cost-effective in order for the implementation of any MAS scheme to be feasible.

A wide array of molecular markers is currently being used in breeding programmes. Although each marker system is associated with advantages and disadvantages the choice of marker system is ultimately chosen, as mentioned above, by the intended application, the marker's reliability, convenience of use and the cost involved. Molecular markers can be broadly classified into three groups: hybridization-based markers such as restriction fragment length polymorphisms (RFLPs); polymerase chain reaction (PCR) based markers such as random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs) or microsatellites and amplified fragment length polymorphisms (AFLPs); DNA micro chips and sequence determination based markers like the single nucleotide polymorphisms (SNPs) and sequence tagged sites (STSs) (Gupta *et al*., 1999; Collard & Mackill, 2008).

The most widely used marker in all major cereals is simple sequence repeat (SSR) (also called microsatellite markers) (Gupta & Varshney, 2000; Collard & Mackill, 2008). SSRs can be found all over the genome of plants and consists of short simple nucleotide sequence tandem repeats. These markers are highly reliable, co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic (Gupta & Varshney, 2000; Collard & Mackill, 2008). However, SSR markers also have disadvantages. Two of the major disadvantages of SSRs are that polyacrylamide gel elecrtrophoresis are required to visualize them and generally give information only about a single locus per assay. In many cases these problems have largely been overcome by means of

selecting SSR markers that have big size differences which allows them to be detected on normal agarose gels; and by means of multiplexing several SSR markers, more than one marker can be screen in a single assay (Collard & Mackill, 2008).

Other markers that are also very useful (and being used in breeding programmes) is sequence tagged sites (STS) and single nucleotide polymorphisms (SNP) markers. These markers are originated from specific DNA sequences which are associated with a gene (Shan *et al*., 1999, Sanchez *et al*., 2000; Sharp *et al*., 2001).

With the use of applicable markers, the breeder has the ability to select for an array of genes that ultimately has the same phenotypic expression, something that he would have not been able to do when relying only on phenotypic expression (Bariana *et al*., 2007). Markers thus have big potential in developing multi-genic resistance lines for specific diseases such as rust. Most of the resistance genes for a specific disease, such as stem rust, have the same phenotypic expression in the field. When breeding for multi-genic resistance for this disease, selecting for lines that carry more than one resistance genes is very difficult. This problem can be overcome by using markers for these genes (Bariana *et al*., 2007). When it comes to economic important recessive traits in wheat, markers has a pivotal role to play in selecting them in early segregating generations. Normally recessive alleles are masked by their corresponding dominant alleles during the heterotic stages of early segregation. They only come into expression after several inbreeding generations after which the recessive alleles may have been lost due to continues selection of the dominant allele (Collard & Mackill, 2008). Markers can help keep important recessive traits in the segregating populations until it comes into expression when the line's homozygous.

The potential advantages of MAS are considerable. As marker technology continues to improve, the incorporation of such technologies gets more feasible to use in breeding programmes; but, when compared to phenotypic screening of traits, the use of molecular markers remains a costly aproach (Marais & Botes, 2009).

2.6. Genes targeted in this study

For this study an array of different rust resistance genes, *Sr31*/*Lr26*/*Yr9*, *Sr24*/*Lr24*, *Sr38*/*Lr37*/*Yr17*, *Sr2*, *Lr34*/*Yr18* and three gluten encoding genes *Glu-Dy10*, *Glu-Dx5* and *Glu-Dy12* were used in conjunction with the dominant male sterility gene *Ms3* in a recurrent mass selection breeding scheme to develop a base population of multi-genic resistance wheat lines.

2.6.1. *Sr31*/*Lr26*/*Yr9* translocation

The 1BL.1RS translocation carrying the linked rust resistance genes *Sr31*/*Lr26*/*Yr9* as well as a powdery mildew resistance gene *Pm8*, has been used in agriculture on the largest scale since the 1980s in spring, facultative and winter wheat breeding programmes worldwide (Singh *et al*., 2011). This translocation was transferred from the short arm of chromosome 1R in rye (*Secale cereale*) to the long arm of chromosome 1B of wheat (McIntosh *et al*., 1995).

The original 1RS translocation from rye, which carried the locus *Sec-1* which encodes the rye storage protein secalin (Shewry *et al*., 1985), replaced the short arm of chromosome 1B which carried several loci that encodes the gluten fraction of the storage protein of wheat (Payne, 1987). The secalin protein causes sticky dough which has a deleterious effect on bread making quality and due to this, this translocation has not been extensively used in South Africa due to its adverse effect it has on wheat quality (Pretorius *et al*., 2007).

This translocation was extensively used internationally because of its near immune-like disease resistance it had to all three rusts pathogens and because of its association with increased grain yield (Lukaszewski, 2000; Singh *et al*., 2011). Large scale deployment since the early 1980s of *Sr31* surprisingly did not result in its breakdown which is normally the case with major resistance gene deployment (Singh *et al*., 2011). It was not until 1998, when the first stem rust susceptible reaction was observed on wheat carrying the 1RS.1BL translocation in Uganda (Pretorius *et al*., 2000). This was a major event because so many wheat cultivars, especially in Asia, were dependent on this resistance source for sustainability (Singh *et al*., 2011). Due to its promise this translocation to disease resistance and yield improvement, considerable work went into developing a 1RS.1BL line without the secalin encoding *Sec-1* locus that cause the sticky dough phenotype (Lukaszewski, 2000).

The 1RS.1BL translocation that will be used in this study is a shortened translocation without the *Sec-1* locus. This modified translocation were developed by inducing several cycles of homoeologous pairing between the chromosome arms 1RS of 'Petkus' rye and 1BS of wheat cultivar 'Pavon' to facilitate recombination between them (Lukaszewski 2000). These homoeologous paring produced chromosomes that cytologically appeared as normal 1RS arms but each has two intercalary segments of 1BS: one introducing the *Gli-1*/*Glu-3* loci and the second one removing the *Sec-1* locus (Lukaszewski, 2000). The protein composition of these modified 1RS.1BL translocation lines was identical to that of normal wheat (Lukaszewski, 2000).

In this study *Sr31* was originally used due to it's near immune disease resistance it provided to the majority of predominant stem rust pathotypes in South Africa (Pretorius *et al*., 2007; Le Maitre, 2010). With the new modified translocation, without the secalin encoding region, it can be used in the strict quality orientated wheat industry of South Africa. Due to the presence of the stem rust pathotype PTKST in South Africa, *Sr31* must not be implemented on its own in a wheat cultivar. PTKST is a very aggressive pathotype and as it spreads, it is estimated that it will become the predominant pathotype (Pretorius *et al*., 2010).

An RFLP probe, iag95, which is located distally to the *Sr31* complex on the chromosome arm, was transferred to a co-dominant STS molecular marker. This marker is being used to track *Sr31* routinely in diverse wheat populations (Mago *et al*., 2002).

2.6.2. *Lr37*/*Yr17*/*Sr38* translocation

The original translocation carrying the complex of linked rust resistance genes, *Lr37*, *Yr17* &*Sr38*, were transferred from *Triticum ventricosum* into the French winter wheat cultivar "VPM1" (Maia, 1967; Helguera *et al*., 2003). The translocation was transferred to the short arm of chromosome 2A (Bariana & McIntosh, 1993). Because of the associated resistance to all three rust pathogens, this translocation was very attractive to wheat breeding programmes (McIntosh *et al*., 1995). Although virulence is recorded for all the three rust resistance genes in this complex, it still provides resistance to an array of different rust pathotypes. This complex is also useful in combination with other rust resistance genes (Helguera *et al*., 2003).

As with other alien gene transfers, this translocation were also transferred into cultivated wheat by means of using the *ph1* gene that promotes homoeologous chromosome recombination. Unlike the

Sr31 complex and the *Lr19* translocation, the *Lr37*/*Yr17*/*Sr38* translocation were not associated with any deleterious quality characteristics or any yield kicks (Helguera *et al*., 2003).

In South Africa virulence is recorded for both *Sr38* and *Yr17*, it is only *Lr37* still effective for the predominant leaf rust pathotypes in South Africa (Pretorius *et al*., 2007; Le Maitre, 2010). *Lr37* provides resistance at the adult plant stage and due to this it is rarely effective at the seedling stage (Sumíková & Hanzalová, 2010). Until 2000, *Sr38* still gave good sustainable resistance to stem rust in South Africa. The stem rust pathotype, 2SA88 acquired virulence for this gene, it became one of the most prominent pathotypes in South Africa up until now (Pretorius *et al*., 2007). Due to this, *Sr38* needs to be used in conjunction with other stem rust resistance genes to ensure sustainable resistance. However, there still is stem rust pathotypes for which *Sr38* is effective (Pretorius *et al*., 2007; Le Maitre, 2010).

Molecular markers have been developed to accelerate the transfer of these genes into commercial cultivars. The specific primers for the complex used in this study, VENTRIUP and LN2, were derived from RFLP probes (Helguera *et al*., 2003).

2.6.3. *Sr24*/*Lr24* translocation

Stem rust resistance gene *Sr24* has been introduced into wheat from *Agropyrum elongatum*. The first cultivar that had this translocation was the Australian cultivar, 'Agent' which carried a spontaneous translocation between chromosome 3Ag of *Agropyrum elongatum* and chromosome 3DL of bread wheat (Smith *et al*., 1968). This 3Ag chromosome segment carried by 'Agent' also carried the leaf rust resistance gene *Lr24*. However, this translocation also had genes encoding for red grain colour (Smith *et al*., 1968). This was not very popular with the Australian and Asian markets and thus, 'Agent', was not over exploited as donor parent in these countries (Mago *et al*., 2005). To reduce the *Agropyrum* chromosome segment, *ph1* mutant background was used to induce homoeologous recombination between wheat and the *Agropyrum* chromosome (Sears, 1973). Several recombinant lines were obtained including white-seeded lines from 3Ag/3D transfers (Sears, 1973). The *Agropyrum* chromosome segment in these recombinant lines was even shorter that the segments derived from the Agent cultivar (McIntosh *et al*., 1995; Friebe *et al*., 1996).

Both *Sr24* and *Lr24* virulence have been recorded in South Africa (Le Roux & Rijkenberg, 1987; Pretorius *et al*., 2007). Due to the intense and widespread production between 1984 and 1987 of the

Agent derived cultivar, SST44, pressure was placed on the highly successful resistant stem rust gene *Sr24*. This led to "the boom and bust phenomenon" and a rapid build up of the *Sr24* virulent stem rust pathotype, 2SA100, which caused great grain losses (Le Roux & Rijkenberg, 1987). Since then *Sr24* were no longer extensively used in breeding programmes. Although when used in combination with other stem rust resistance genes, in South Africa it still can give sustainable protection since some of the stem rust pathotypes still is avirulent for *Sr24* (Pretorius *et al*., 2007).

The sequenced characterized amplified region (SCAR) marker, $SCS73_{719}$, is very closely linked to *Lr24*. Due to this, the translocation carrying the linked gene *Sr24*/*Lr24* can be traced in wheat populations. The marker was originally developed for *Lr19* from a RAPD ($S73_{728}$) (Cherukuri *et al*., 2003). But later it was found that this marker is associated with *Lr24* rather than with *Lr19* (Prabhu *et al*., 2004). This happened since genes originated from a translocation form *Agropyrum* and *Lr24* were mistaken for *Lr19* (Prabhu *et al*., 2004).

2.6.4. *Lr34*/*Yr18* complex

Lr34/*Yr18* has provided durable resistance to both leaf and stripe rust (Dyck *et al*., 1966; Singh & Rajaram, 1992; Ma & Singh, 1996). *Lr34* is a durable rust resistance gene complex which protects wheat against complete infestation of leaf rust (Singh *et al*., 2005; Spielmeyer *et al*., 2005). It was found that *Lr34* even can provide sufficient seedling resistance to some pathotypes of leaf rust (Singh *et al*., 2005). This gene gives a broad-spectrum resistance to a wide array of leaf rust pathotypes in the adult plant stage. The working of the resistance mechanism is through lengthening the latent period of the rust infection and also hampering the growth and development of haustoria, which ultimately causes a reduction in the number and size of the uredinia on the green tissue (Singh *et al*., 2005).*Yr18* also has a durable resistance reaction, as *Lr34*, by means of lengthening the latent period in stripe rust development and decreasing the infection frequency and the overall length of the lateral orientated uredinia lesions on adult plant leaves (Singh *et al*., 2005). *Lr37* and *Yr18* are very closely linked with each other on the short arm of chromosome 7D (Singh, 1992; McIntosh, 1992; Dyck, 1987).

Due to the partial resistance reaction these linked gene provides for leaf and stripe rust, it is wise to implement them in conjunction with other resistance genes to provide sufficient protection under heavy epidemics conditions (Singh *et al*., 2005).

This durable rust resistance source can be selected by using a phenotypic marker which is co-inherited with *Lr34*/*Yr18*. This phenotypic marker which is visualized as necrosis of the tips of the flag leaves is conveyed by a gene that expresses leaf tip necrosis (LTN).This gene is closely linked to *Lr34*/*Yr18* which makes it useful to use as phenotypic marker (Singh *et al*., 2005; Spielmeyer *et al*., 2005).

The problem with LTN and numerous other phenotypic markers is the large genotype by environment interaction accompanying its expression (Spielmeyer *et al*., 2005).

Genetic studies of *Lr34*/*Yr18* were done and molecular markers have been developed to trace and to facilitate the incorporation of *Lr34*/*Yr18* into breeding populations (Spielmeyer *et al*., 2005; Lagdudah *et al*., 2006; Krattinger *et al*., 2009). Krattinger *et al*. (2009) were the first to successfully clone the gene complex. It was found that a single gene complex is responsible for the resistance that is based on *Lr34* and *Yr18*.The cloning of *Lr34*/*Yr18* helped in the development in a diagnostic marker that amplifies a region specifically inside the *Lr34*/*Yr18* gene complex (Krattinger *et al*., 2009). This marker, being implemented in a multiplex together with linked markers can increase the selection of *Lr34*/*Yr18* substantially (Wessels, 2010).

2.6.5. *Sr2* translocation

Sr2 is a broad-spectrum durable resistance gene for stem rust in wheat (Knott, 1968). *Sr2* is arguably the most important gene for stem rust resistance employed in wheat breeding (McIntosh *et al*., 1995). *Sr2* has been effective against stem rust since its transfer from tetraploid emmer wheat cultivar ‘Yaroslav’ into the bread wheat cultivar ‘Marquis’ in the 1920s (McFadden, 1930; McIntosh *et al*., 1995; Mago *et al*., 2011). The cultivar ‘Hope’ was the first *Sr2* carrying agronomical acceptable cultivar to be released from this interspecific cross (Borlaug, 1968). *Sr2* is effective in the adult plant stage against all known pathotypes of stem rust including the aggressive stem rust pathotype Ug99 which has virulence to many important resistance genes, including *Sr31*, *Sr24* and *Sr38* (Pretorius *et al*., 2000; Pretorius *et al*., 2010). As with the broad-spectrum resistant genes *Lr34*/*Yr18*, the partial resistance encoded by *Sr2* on its own also provides insufficient protection under prolonged disease pressure. Due to this, *Sr2* needs to be employed into wheat cultivars along with other resistance genes. The moderate resistance response and recessive gene action of *Sr2* makes field selection very difficult for this durable stem resistance gene (Mago *et al*., 2011).

Sr2 is located on the short arm of chromosome 3B (Knott 1968; McIntosh *et al*., 1995). As with *Lr34*/*Yr18*, *Sr2* also has a phenotypic marker closely linked to it that can be used as a phenotypic marker in breeding populations. The phenotypic marker is pseudo black chaff (PBC), which is a dark pigmentation that develops around the stem internodes and glumes (McIntosh *et al*., 1995). Because the level of PBC expression is dependent on environmental conditions and genetic background effects, the reliability of PBC as marker across a range of environments is questionable (Spielmeyer *et al*., 2003). Due to the difficulty associated with field selection of *Sr2* molecular markers have been developed to facilitate breeding programmes in breeding for *Sr2* resistance (Mago *et al*., 2011). Several molecular markers have been developed for selecting for *Sr2* in wheat breeding populations (Spielmeyer *et al*., 2003; Hayden *et al*., 2004; McNeil *et al*., 2008; Mago *et al*., 2011). Most recently CAPS marker was found to be associated with *Sr2* in 95% of very diverse *Sr2* carrying genotypes. The marker also discriminated 100% against a very diverse group of genotypes not carrying *Sr2*, something the previous markers could not detect (Mago *et al*., 2011).

2.6.6. HMW-GS genes

The HMW-GS are key components of the glutenin polymer and therefore play an essential role in determining the visco-elastic properties of wheat quality (Payne *et al*., 1987). The interaction between the different encoded HMW-GS has an additive effect when investigating dough properties (Beasley *et al*., 2002).

The HMW-GS are encoded by genes, collectively called *Glu-1* loci, which is present on the homoeologous group 1 chromosomes of hexaploid wheat. These loci, *Glu-A1*, *Glu-B1* & *Glu-D1*, are situated in close proximity to the centromeres on the long arms of chromosomes 1A, 1B and 1D (Payne & Lawrence, 1983). Each of the three loci, encoding the HMW-GS, is composed out two tightly linked genes, which are always inherited as a complex (Galili & Feldman, 1983; Lawrence & Shepherd, 1981). At each of the three *Glu-1* loci the two tightly linked HMW-GS genes encode for different HMW-GS known as an x- and a y-type subunit. The x-type subunit gene encodes a HMW-GS which is of a higher molecular weight than the HMW-GS encoded by the y-type subunit gene (Payne *et al*., 1981; Payne & Lawrence, 1983). Thus in theory hexaploid wheat could contain six different subunit encoding genes which is comprised from the two linked genes from each of the three loci, *Glu-A1*, *Glu-B1* &*Glu-D1*. But in reality, only three, four or five subunits are present in a bread wheat cultivar which is caused by gene inactivation (Galili & Feldman, 1983; Shewry *et al*.,

1992). The genes are co-dominant which entails that products of each gene being expressed are present in the grain endosperm (Payne *et al*., 1987).

Genes encoding HMW-GS are expressed in a highly regulated manner in the developing endosperm of the wheat seed. As mentioned, hexaploid wheat contain up to five copies of these genes, which are located in pairs on group 1 of all three homoeologous chromosomes 1A, 1B and 1D. These gene pairs are made up of an x-type and y-type HMW encoding subunit gene. In hexaploid wheat, the y-type subunit encoding gene on the A chromosome is not usually expressed (Payne *et al*., 1981; Reddy & Apples, 1993). For the loci, *Glu-B1* &*Glu*-D1, x- and y-type HMW encoding subunit genes are normally both expressed in wheat (Payne *et al*., 1981).

Each of the three loci exhibits extensive allelic variation and polymorphism. These allelic variation found at each of the three loci is due to successive mutation events for both of their x- and y-type subunit encoding genes (Payne *et al*., 1981, Galili & Feldman, 1983; Payne *et al*., 1987). The specific allelic composition of the HMW-GS is one of the most imperative genetic factors which determine the dough-forming properties of a wheat cultivar (Payne *et al*., 1987). For visualization purposes each of the three loci and some of their most common and important alleles are summarized in Table 2.4.

Table 2.4. Bread quality scores and their corresponding *Glu-1* alleles (Payne *et al*., 1987).

Glu-A1			***Glu-B1***			***Glu-D1***		
Score	**Allele**	**Subunits**	**Score**	**Allele**	**Subunits**	**Score**	**Allele**	**Subunits**
3	*A*	1	3	*i*	17 +18	5	*d*	5 + 10
3	*B*	2*	3	*b*	7 +8	2	*a*	2 + 12
1	*C*	Null	2	*c*	7 + 9	2	*b*	3 + 12
-	-	-	1	*a*	7	1	*c*	4 + 12
-	-	-	1	*d*	6 + 8	-	-	-

It is important to note that these different alleles have additive, as well as, interactive effects with each other (Payne *et al*., 1987). Thus when breeding for quality, by means of actively selecting for HMW-GS, the additive and interactive effects needs to be taken in consideration when selecting breeding populations (Radovanovic & Cloutier, 2003).

For the A genome subunits, breadmaking quality of wheat cultivars in respect to HMW-GS increased in the direction of null <1 < 2* (Moonen *et al*., 1983). It was found that chromosome 1A encoded subunits 1 and 2* were associated with large sodium dodecyl sulphate (SDS) sedimentation values (SDS sedimentation is an indicator of bread making quality) (Payne *et al*., 1981).

In particular, the *Glu-D1* locus with its allelic compositions has a major determining effect on dough strength. Furthermore with the allelic variation of the *Glu-D1* locus, wheat containing the *d* allele (Dx5 paired with Dy10 HMW-GS) has an inherent stronger dough than wheats containing the *a* allele (Dx2 paired with Dy12 HMW-GS) (Greene *et al*., 1988; Lafiandra *et al*., 1993).

To evaluate a specific wheat cultivar or a group of cultivars glutenin polypeptide profiles, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is used to visualize their specific allelic variation. These allelic variations are depicted by specific banding patterns on the gel (Payne *et al*., 1980). This method is relatively efficient and is advantageous because allelic variation at multiple loci can be assessed in a single gel lane. However, it requires seeds for extraction of protein and thus cannot be used as selection method during early plant development phases. Evaluating and scoring SDS-PAGE banding patterns also needs technical expertise to describe the different bands allocated to different subunits. Some of the bands of different subunits also lie very close to each other on the gel (Gale, 2005). To address these problems molecular markers have been developed for an array of alleles of all three *Glu-1* loci (Radovanovic & Cloutier, 2003; Gale, 2005). Molecular markers also can distinguish among alleles encoding for very similar subunits, for instance the two alleles of the *Glu-B1* locus encoding subunits 7 and 7*. On the SDS-PAGE gel these two subunits would not be able to be distinguished from each other, but molecular markers is available to distinguish between their two alleles (Radovanovic & Cloutier, 2003; Lei *et al*., 2006).

3. Materials and Methods

In order to achieve the aim of this project in identifying and evaluating superior rust resistant crossing parents out of an existing MS-MARS population of the SU-PBL, the following three objectives were followed as depicted in Table 3.1. Firstly segregating material from an existing MS-MARS base population developed by Marais & Botes (2003) was screened by means of MAS for rust resistance genes and gene complexes. This MS-MARS base population were the result of numerous RMS cycles for progeny developed by combining high yielding good agronomical characteristic parents with good rust resistant parents (Marais & Botes, 2009). Secondly the MAS selected lines were made pure breeding by means of the wide hybridization doubled haploid method. Thirdly the pure breeding lines were put through another MAS cycle to confirm the presence of the originally selected genes. The pure breeding lines were then subjected to seed multiplication phases to acquire enough seed for an extensive multi-location field evaluation trial where yield potential and bread making quality characteristics were evaluated. Adult plant resistance to stem, leaf and stripe rust were also evaluated at two localities that has climatic conditions which can help with disease development (Table 3.1).

Table 3.1. Schematic overview of the MSc study.

DEVELOPMENT OF MS-MARS POPULATION	***Female parents***	***Male parents***	2003-2008	Use male sterile (*Ms3ms3*) lines out of a 1:1 segregating F1 population and MAS selected male parents (Marais & Botes, 2003)
	Ms3ms3 and segregating for *Sr38*-	*Sr31*-complex and *Lr19*		
	Male sterile lines	MAS and seedling rust testing		Mass crossing between female (*Ms3ms3*) and MAS selected male parents by means of hydroponic tiller scheme (Marais & Botes, 2003)
	Random crossing using hydroponic tiller scheme			
1. MSC PROJECT: MAS	MAS material out of existing MS-MARS population for *Sr31*-complex, *Lr19*, *Sr38*-complex and *Sr24*/*Lr24* Select MS-MARS lines with more multi-genes for rust resistance		2008	Use of MAS to screen a subset of 64 MS-MARS lines for the presence of the different molecular markers associated with their respective rust resistance genes
2. MSC PROJECT: DH, SEED MULTIPICATION & MAS	Develop DH lines from MAS selected MS-MARS F1 progeny		2008-2009	Selected MS-MARS lines that amplified multiple markers, associated with the resistance genes, were selected and made pure breeding by means of the DH method
	Seed multiplication phase		2009-2011	MS-MARS inbred lines carrying the multi-resistance genes were incorporated into a seed multiplication phase
	Determine genotype profile of DH MS-MARS lines by MAS for *Sr31*-complex, *Sr38*-complex, *Sr24*/*Lr24*, *Sr2,Lr34*/*Yr18*&*Glu*-genes		2011	Use of MAS to screen MS-MARS inbred lines for the presence of the different molecular markers associated with their respective rust resistance and HMW-GS genes
3. MSC PROJECT: Multi-environment trial	Multi-location yield and rust resistance screening		2011-2012	After seed multiplication the MS-MARS inbred lines were incorporated into a multi-location yield trial and rust screening to evaluate their adult plant rust resistance, adaptability, stability, yield potential and quality attributes

3.1. MAS of multi-rust resistance genes carrying lines from MS-MARS population

3.1.1. Plant material

Plant material used in this study came from segregating material sourced from a highly heterogenous(pre-breeding) wheat population from the SU-PBL. This population was developed by employing male sterility mediated marker-assisted recurrent selection (MS-MARS) (Marais & Botes, 2009). This technique uses recurrent selection in wheat by means of employing the dominant male sterility gene *Ms3* so that mass crossings could be facilitated between mass selected wheat lines. The MS-MARS population was developed by the SU-PBL (Marais & Botes, 2003) and it segregated for an array of different rust resistance genes. Of the rust resistance genes/complexes that segregated in the MS-MARS population were *Sr31*/*Lr26*/*Yr9*, *Lr24*/*Sr24*, *Lr37*/*Sr38*/*Yr17*, *Lr34*/*Yr18* and *Sr2*.The plant material used in this study came from a subset of 64 lines which were sourced from the SU-PBLs pre-breeding MS-MARS population. This population was developed by using a male parent group carrying the rust resistance complex *Sr31*/*Lr26*/*Yr9*, and a female parent group which were heterozygous for the *Ms3* gene (*Ms3ms3*) and carried the rust resistance genes; *Lr24*/*Sr24*, *Lr37*/*Sr38*/*Yr17*, *Lr34*/*Yr18* and *Sr2*. The initial phases of the development of this MS-MARS population, involved a pedigree breeding approach where male lines which had good rust resistance were put through agronomic and yield evaluations. Good agronomic and high yielding male material was cycled back through the MS-MARS population to enrich these important characteristics in the MS-MARS population. By employing these male parents in the mass crossings, the highly heterotic MS-MARS population had good agronomic and yield adaptability circulated through it on a continuous basis (Marais & Botes, 2003).

The subset of 64 MS-MARS lines were planted in a greenhouse at Welgevallen experimental farm, Stellenbosch University, Stellenbosch, South Africa to acquire fresh leave tissue to subject them all for MAS.

3.1.2. Marker-assisted selection for the rust resistance genes

3.1.2.1. Genomic DNA extraction and quantification

An adaptation of Doyle & Doyle (1990) was used for the extraction of genomic DNA (gDNA) from the 64 MS-MARS lines that was planted in the greenhouse at Welgevallen. For each MS-MARS line a leaf piece of 2 to 4cm, in length, was cut into small pieces and placed into a 2.2 ml micro centrifuge tube, containing two small sterilized stainless steel balls. Eight hundred micro litres of 2% (m/v) cetyltrimethylammonium (cetrimonium) bromide (CTAB) [1.4 M NaCl, 20mM EDTA (pH 8.0), 100mM Tris-Cl (ph 8.0)] and 1.6 µl 0.2% (v/v) beta-mercaptoethanol (BME) were added to tube containing the leaf tissue. The leaf tissue and CTAB mixture were then grinded using a Qiagen® TissueLyser (Qiagen, Southern Cross Biotech, Claremont, RSA) for more than 90 seconds at a frequency of 30 Hz. After each 90 second cycle, the tubes were rotated. After the grinding process the mixture was incubated in a 60˚C water bath for 60 minutes. Following incubation 800 µl chloroform: isoamyl alcohol (24:1) was added to the mixture and centrifuged for 8 minutes at 12,000 rpm. Approximately 600 µl of the supernatant was transferred to a clean 2.2 ml microcentrifuge tube where 600 µl phenol: chloroform: isoamyl alcohol (25:24:1) was added. The mixture was then centrifuged for 3 minutes at 12,000 rpm. Approximately 550 µl of the supernatant were transferred to a clean 2.2 ml tube where 550 µl chloroform: isoamyl alcohol (24:1) was added after which it was centrifuged for a further 5 minutes at 12,000 rpm. The supernatant was then transferred to a clean 1.5 ml microcentrifuge tube after which cooled (4˚C) isopropanol of the same volume as supernatant were added. This mixture was then incubated overnight at -20˚C and centrifuged the next morning for 10 minutes at 4˚C at a speed of 12,000 rpm. After centrifugation, care was taken when the isopropanol was discarded in order to discard of the DNA pellet. The pellet was then washed by centrifuging the pellet with 70% (v/v) ethanol at 4˚C (6 minutes at 12,000 rpm). The ethanol supernatant was discarded and the DNA pellet was left to air dry. The DNA pellet was subsequently dissolved in 50 µl Tris-EDTA (pH 8.0) and 40 µg/ml RNase A and incubated for 30 minutes at 37˚C. After incubation 0.1 volume sodium acetate (NAOAc) (3 M, pH 5.0) and two volumes 100% ethanol was added to the mixture and centrifuged for 10 minutes (4˚C, 12,000 rpm). The DNA pellet was then washed two times with 70% (v/v) ethanol for 6 minutes (4˚C, 12,000 rpm). Afterwards the pellet was left to dry and resuspended in 30 µl ddH_2O and stored at 4˚C.

The concentration of the extracted gDNA of each of the 64 MS-MARS lines were determined using a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Kempton Park, RSA). All the samples that were used for molecular marker screening were diluted using ddH_2O to a final concentration of 50ng/µl.

3.1.2.2. Molecular markers-assisted selection

The primers used to screen for the genes of interest are given in Table 3.2. All except for the *Lr24*/*Sr24* and *Lr37*/*Sr38*/*Yr17* complexes were amplified individually by using the PCR conditions given below and the cycling conditions in Table 3.3.

PCR amplification of the specific markers were performed in a 20 µl reaction mixture containing Kapa Biosystems® KapaTaq Readymix® DNA Polymerase (2X, 0.05U.µl^{-1} with Mg^{2+}, 0.4mM of each dNTP plus loading dye), primer (Table 3.1.2.2.1), and ± 50ng of DNA.

With the onset of the study the subset of 64 MS-MARS lines, the molecular markers were used to screen for the three rust resistance gene complexes *Sr31*/*Lr26*/*Yr9*, *Lr24*/*Sr24* and *Lr37*/*Sr38*/*Yr17*. The original MS-MARS population developed by Marais & Botes (2003) also carried the rust resistance genes *Lr34*/*Yr18* and *Sr2*. In this study these genes were first screened for after the MS-MARS lines were made pure breeding following the DH technique when efficient molecular markers became available.

Table 3.2. Primers and PCR characteristics of each resistance gene marker used in this study.

Gene	Primer name	Sequence	Annealing temperature	Approx Fragment size	Screen for	Reference
Sr31/Lr26/Yr9	Iag95-F	FP: 5' CTC TGT GGA TAG TTA CTT GAT CGA 3'	52°C	1000bp	Rust genes	Mago *et al*., 2002
	Iag95-R	RP: 5' CCT AGA ACA TGC ATG GCT GTT ACA 3'				
Lr24/Sr24	SCS_{719}-F	FP: 5' TCG TCC AGA TCA GAA TGT G 3'	55°C	719bp	Rust genes	Cherukuri *et al*., 2003
	SCS_{719}-R	RP: 5' CTC GTC GAT TAG CAG TGA G 3'				
Lr37/Sr38/Yr17	VENTRIUP-F	FP: 5' AGG GGC TAC TGA CCA AGG CT 3'	65°C	259bp	Rust genes	Helguera *et al*., 2003
	LN2-R	RP: 5'TGC AGC TAC AGC AGT ATG TAC ACA AAA 3'				
Lr34/Yr18	L34DINT9-F	FP: 5' TTG ATG AAA CCA GTT TTT TTT CTA 3'	58°C	517bp	Rust genes	Krattinger *et al*., 2009
	L34PLUS-R	RP: 5' GCC ATT TAA CAT AAT CAT GAT GGA 3'				
Sr2	*cs*SR2-F	FP: 5' CAA GGG TTG CTA GGA TTG GAA AAC 3'	60°C	172bp or 225bp	Rust gene	Mago *et al*., 2011
	*cs*SR2-R	RP: 5' AGA TAA CTC TTA TGA TCT TAC ATT TTT CTG 3'				
Glu-Dy10	P3	FP: 5' GTT GGC CGG TCG GCT GCC ATG 3'	63°C	576bp	HMW-GS	Ahmed,2000
	P4	RP: 5' TGG AGA AGT TGG ATA GTA CC 3'				
Glu-Dx5	P1	FP: 5' GCC TAG CAA CCT TCA CAA TC 3'	63°C	450bp	HMW-GS	Ahmed,2000
	P2	RP: 5' GAA ACC TGC TGC GGA CAA G 3'				
Glu-Dy12	P3	FP: 5' GTT GGC CGG TCG GCT GCC ATG 3'	63°C	612bp	HMW-GS	Ahmed,2000
	P4	RP: 5' TGG AGA AGT TGG ATA GTA CC 3'				

Table 3.3. Optimal PCR cycling conditions for detection of the six rust resistance genes/gene complexes and HMW-GS genes used in this study.

Sr31/Lr26/Yr9		***Lr24/Sr24 &Lr37/Sr38/Yr17***		***Lr34/Yr18***		***Sr2***		***Glu-Dy10***		***Glu-Dx5***		***Glu-Dy12***	
95°C	3 min	94°C	4 min	94°C	5 min	95°C	2 min	94°C	5 min	94°C	5 min	94°C	5 min
95°C	30 sec	94°C	1 min	94°C	1 min	95°C	30 sec	94°C	1 min	94°C	1 min	94°C	1 min
52°C	30 sec	60°C	1 min	58°C	1 min	60°C	40 sec	63°C	1 min	63°C	1 min	63°C	1 min
72°C	50 sec	72°C	1 min	72°C	1 min	72°C	50 sec	72°C	1 min	72°C	1 min	72°C	1 min
72°C	10 min	72°C	7 min	72°C	5 min	72°C	5 min	72°C	10 min	72°C	10 min	72°C	10 min
4°C	∞	4°C	∞	4°C	∞	4°C	∞	4°C	∞	4°C	∞	4°C	∞
35 cycles		35 cycles		35 cycles		30 cycles		45 cycles		45 cycles		45 cycles	

3.2. Creating inbred lines from marker-assisted MS-MARS lines

3.2.1. Double haploid production

The development of DH plants was done as part of the SU-PBLs DH programme which followed the protocol of Pienaar *et al*.(1997). It involved crossing the emasculated ears of the MAS selected MS-MARS lines with maize pollen and treating each floret 30 hours after pollination with a growth regulating solution made up of 50mg/L 2,4-D and 100mg/L GA_3. Embryos of each MS-MARS line were rescued 18 – 21 days afterwards and placed in tissue bottles containing growth medium. Haploid embryos which had developed into plants were transplanted to pots filled with peat. At three leaf stage the haploid plants were subjected to a 0.05% (w/v) colchicine solution in order to double it's haploid number.

3.2.2. Seed multiplication phase

The MS-MARS lines that were made pure breeding through the SU-PBL's DH programme were planted out in the greenhouse. From the greenhouse the pure breeding MS-MARS lines were incorporated into the SU-PBLs field nurseries for two years (4 cycles) to increase the seed of each line to such an extent that enough seed (1280g) were available for extensive field evaluation purposes. Each of the pure breeding MS-MARS lines were also subjected to gDNA extraction and screening with the updated 2011 SU-PBL marker panel to have a genotype profile constructed of them.

3.3. Field evaluation and final selection for crossing parents from MS-MARS lines

3.3.1. Multi-environment yield trials for agronomic and quality characteristic evaluations

During the wheat production season of 2011, the MS-MARS inbred lines and five checks (Table 3.4) were planted in a multi-environment yield trial (Figure 3.1). The trial comprised four experiments, each planted at a different location in South Africa. In order to evaluate adaptability

and stability three dryland wheat production areas in the Western Cape were used to represent low to marginal wheat production environments and one area in the Northern Cape, representing high input irrigation conditions (Table 3.5).

Figure 3.1. Map of South Africa indicating the four locations where the experiments were planted.

Table 3.4. List of commercial cultivars and MS-MARS lines planted in each of the four experiments.

Entry	Name	Source	Information
1	SST056	Sensako (Pty.) Ltd.	Dryland spring wheat cultivar
2	SST047	Sensako (Pty.) Ltd.	Dryland spring wheat cultivar
3	SST806	Sensako (Pty.) Ltd.	Irrigation spring wheat cultivar
4	SST867	Sensako (Pty.) Ltd.	Irrigation spring wheat cultivar
5	US 1010	SU-PBL	Dryland spring wheat cultivar
6	MS-MARS-06	SU-PBL MS-MARS	Multi-genetic resistance line
7	MS-MARS-07	SU-PBL MS-MARS	Multi-genetic resistance line
8	MS-MARS-08	SU-PBL MS-MARS	Multi-genetic resistance line
9	MS-MARS-09	SU-PBL MS-MARS	Multi-genetic resistance line
10	MS-MARS-10	SU-PBL MS-MARS	Multi-genetic resistance line
11	MS-MARS-11	SU-PBL MS-MARS	Multi-genetic resistance line
12	MS-MARS-12	SU-PBL MS-MARS	Multi-genetic resistance line
13	MS-MARS-13	SU-PBL MS-MARS	Multi-genetic resistance line
14	MS-MARS-14	SU-PBL MS-MARS	Multi-genetic resistance line
15	MS-MARS-15	SU-PBL MS-MARS	Multi-genetic resistance line
16	MS-MARS-16	SU-PBL MS-MARS	Multi-genetic resistance line
17	MS-MARS-17	SU-PBL MS-MARS	Multi-genetic resistance line
18	MS-MARS-18	SU-PBL MS-MARS	Multi-genetic resistance line
19	MS-MARS-19	SU-PBL MS-MARS	Multi-genetic resistance line
20	MS-MARS-20	SU-PBL MS-MARS	Multi-genetic resistance line

Table 3.5. Locations used for yield trials for testing the lines agronomical and quality characteristics during the 2011 season.

Location	Location description	Traits		GPS coordinates
		Yield	Quality	
Langgewens	Dryland, marginal to medium potential	X	X	33°16’27.1”S 18°42’44.2”E
Welgevallen	Dryland, low to marginal potential	X	X	33°56’37.3”S 18°51’55.1”E
Tygerhoek	Dryland, marginal to high potential	X	X	34°09’24.5” S 19°54’27.9”E
Hartsvallei	Irrigation, High potential	X	X	28°00’46.2” S 24°44’55.9” E

In each of the experiments, five checks were included to serve as comparative controls which the lines could be evaluated against. Of these checks four were sourced from Sensako (Pty.) Ltd. and one was an advanced breeding line from the SU-PBL (Table 3.4). Each experiment was planted as a randomized complete block design with four replications (Langgewens, Welgevallen and Tygerhoek) and three replications (Hartsvallei). The yield trials had experimental plots which were 5 m in length and consisted of 6 rows with 17 cm inter-row spacing. The experimental plots were planted with an experimental plot spider (Petrus Steyn Engineering works, Petrus Steyn, South Africa). Each plot was planted at a seed density of 110 kg/ha. Fertilizer application varied between localities. At the experiments planted at Langgewens, Welgevallen and Tygerhoek, 46 kg nitrogen per hectare fertilizer were applied at planting through the planter's fertilizing apparatus. The head dressing, which occurred 40 days after planting, were done manually using the same fertilizer formulation of 46 kg Nitrogen per hectare. At Hartsvallei a total amount of 240 kg/ha nitrogen was applied, using a fertilizer mixture of 8:2:3 (40). Standard agronomic practices were followed in applying this amount through the season. The irrigation schedule at Hartsvallei depended on the field water capacity of the soil. Regular soil moisture readings were taken to keep the field water capacity on an acceptable level so that the plants did not go into any kind of water related stress.

After physiological ripening, the plants were given sufficient time for drying off before harvesting commenced in late October at Langgewens, Welgevallen and Tygerhoek and beginning of December at Hartsvallei. Harvesting was done with the Nursery Master Elite and the Nursery Master Expert (Wintersteiger®, Austria) experimental plot combines. Each of the individual experimental plots was harvested into separate harvest bags.

The harvested grain was weighed and each plot's yield (in grams) was recorded. Tags attached to the bags facilitated in identifying each individual harvest bag's contents to its corresponding entry. After the weighing was concluded, each plot's yield were converted to tons per hectare and used as the official grain yield value that was used in the trial data analysis. The grain was subsequently cleaned of any debris and chaff which remained after harvesting and re-sealed and stored in a cool dry place. Samples were sourced from each of the harvesting bags as needed and used for quality analysis.

3.3.2. Trial data analysis

The trial was conducted over two mega-environments in South Africa, namely the dryland winter rainfall region (Langgewens, Welgevallen and Tygerhoek), and the irrigation summer rainfall region (Hartsvallei). For each of the experiments a general linear model (GLM) analysis, as well as, a nearest neighbour analysis (NNA) were performed to evaluate each experiment's coefficient of determination (R^2) and broad sense heritability (H^2) calculated using Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada). Parameters from both the GLM and NNA were compared in order to evaluate each model's coefficient of determination and heritability parameters. The R^2 indicates how well an regression line can fit when it is plotted between the estimated data, obtained through the model's equation, and the the actual data obtained from the actual yields. When the R^2 is low, estimated data and actual yield data do not correlate well, while when the R^2 is high, estimated data and actual yield data correlates very well indicating a very good model fit. The H^2 reflects the genetic contribution to the total phenotypic variance which is influenced by additive, dominant and epistatic (multi-genic interactions) effects. The higher H^2 is, the more reliable selection will be due to that the majority of the variance is explained through genetic effect of the lines being tested. The yield estimates of each line that came from the analysis with the best R^2 or H^2 were used in a multi-location analysis. In order to evaluate the adaptability and stability of each of the lines over the mega environments, the additive main effects and multiplicative interaction (AMMI) analysis was implemented to analyze the combined estimated data from each of the experiments. This model combines the additive main components, obtained from the combined ANOVA, as well as, the multiplicative components obtained through a principal component analysis (PCA) (Van Eeuwijk, 1995; Crossa *et al*., 1991).

3.3.3. Evaluating MS-MARS lines quality characteristics and determining the *Glu-1* score of each check and MS-MARS line

The quality characteristics evaluated for each of the MS-MARS lines were divided into four broad based quality groups namely industrial quality, physical seed quality, rheological quality and baking quality (Table 3.6).

Quality analyses on all the inbred lines were done by the wheat quality laboratory of Sensako (Pty) Ltd. in Bethlehem. Samples were taken from all four experiments (each replication) and evaluated

for hectolitre mass, kernel hardness, thousand kernel mass, and kernel diameter. The samples were then conditioned (American Association of Cereal Chemists (AACC) method 26-95) and milled after which the milling characteristics and flour yield were determined. Milled flour samples were used to determine flour protein content, falling number and rheological characteristics. Bread baking characteristics of all these samples were also evaluated in the experimental baking laboratory. The mean of each line's quality characteristics at each of the four experiments were calculated by pooling all the replications and calculating the average for each characteristic. To evaluate the overall quality of each entry, a method was used developed by the South African Grain Laboratory (SAGL) which compares each entry's pooled quality characteristics to that of the quality standard, 'SST806' (SAGL, 2010).

Table 3.6. The four broad quality groups tested for in this study.

Quality group	Quality characteristics	Method/Apparatus
Industrial quality	Hectolitre mass (HLM)	Two-level funnel
	Break Flour Yield (BFY)	Chopin® CD1 mill
	Total Flour Extraction (EX)	Chopin® CD1 mill
	Falling Number (FN)	Perten® Falling Number 1700
	Protein content (PROT)	Foss® Infratec 1241
Physical quality	Seed Hardness (HI)	SKCS® 4100
	Thousand Kernel Mass (TKM)	SKCS® 4100
	Seed Diameter (DIAM)	SKCS® 4100
Rheological quality	Dough Mixing Time (PT)	Mixograph & Mixsmart
	Flour Water Absorption (ABS)	Mixograph & Mixsmart
Baking quality	Bread Volume (VOL)	Experimental small scale bake test

The following quality determination methods were employed:

3.3.3.1. Hectolitre mass (AACC method 55-10)

Hectolitre mass was determined by using a two-level funnel. Hectolitre mass was calculated by dividing the obtained mass (in grams) by five. The values expressed were in kg/hl.

3.3.3.2. Kernel characteristics (AACC method 55-31)

The HI, TKM and DIAM were determined by using the Single Kernel Characterization System 4100 (SKCS 4100). Data were generated by means of sending 50 kernels of each sample through the SKCS. Mean values of all the pooled data per sample were obtained and used in the analysis.

3.3.3.3. Break flour yield (AACC method 26-21A)

Each of the whole kernel samples of the MS-MARS lines and conditioned for 24 hours prior to milling. This was done in accordance to the AACC (2000) procedure 26-95. Wheat samples were milled on a laboratory, pneumatic mill, Chopin© CD1 flour mill. The percentage of BFLY was determined for each of the samples using a simple total percentage formula:

% BFLY = [Total 1st break flour obtained / Total conditioned grain] X 100

3.3.3.4. Extraction (AACC method 26-21A)

The total EX of each sample was determined as follows:

% EX = [Total flour obtained / Total conditioned grain] X 100

3.3.3.5. Protein content (AACC method 46-30)

PROT was determined by the Foss® Infratec 1241 Grain Analyzer on milled white flour.

3.3.3.6. Falling number (AACC method 56-81B)

To measure the α-amylase activity of each of the MS-MARS lines, the Perten® Falling Number 1700 apparatus were used. The FLN is determined by an apparatus that measures the time it takes a metallic stirrer to fall through a heated well mixed flour and water suspension. To take the altitude effects into consideration, altitude-corrected values were used to visualize final measurements (AACC, 2000).

3.3.3.7. Mixograph analyses (AACC method 54-40A)

To evaluate PT and ABS of the MS-MARS lines a 35 g flour mixograph test was performed on each sample. The mixograph of each was visually evaluated by Mixsmart software. In order to perform a mixograph test the protein and moisture content of each sample first needed to be determined to calculate the flour weight and water volume required (Walker *et al*., 1997).

3.3.3.8. Experimental bread baking test (AACC method 10-10.03)

To evaluate each line's bread baking quality the optimized straight-dough bread making method was followed. It involved baking bread from 100g of flour and measuring it's volume. Some modifications to the original method were excluding the potassium bromate ($KBrO_3$) solution and replacing the malt extract with 0.25g dry malting barley flour.

HMW-GS were screened by the Sensako quality laboratory using the Agilent® 2100 Bioanalyzer high throughput microchip capillary electrophoresis-sodium dodecyl sulphate platform, Protein 230 Kit (Anatech® Analytical Technologies, Johannesburg, South Africa). The protocol that was followed was that of Uthayakumaran *et al*.(2006). It involved the extraction and purification of each entry's protein by making use of the Agilent® Protein 230 Kit. Each entry's protein sample were loaded onto a primed 230 Protein LabChip for further protein evaluation. Each entry's HMW-GS was visualized with the Agilent® 2100 Bioanalyzer lab-on-a-chip 2100 Expert software (Agilent® Technologies, Palo Alto, California, USA).

The HMW-GS scores were calculated and compared to each of the MS-MARS line's overall quality.

3.3.5. Field rust inoculation and adult plant resistance evaluation

To ensure an effective rust evaluation of resistance to all three rust pathogens, two localities were identified. It included Bethlehem in the eastern Free State and Makhathini in northern KwaZulu-Natal (Figure 3.2 & Table 3.7). The field rust evaluation spanned over two years from 2011 to 2012.

Figure 3.2. Map of the eastern part of South Africa indicating the two locations where the MS-MARS lines were screened for adult plant resistance to the three rust pathogens.

Makhathini is located in the warm north eastern part of KwaZulu-Natal, situated 60 meters above sea level and about 50 kilometres from the warm Indian Ocean. This site was specifically chosen for the evaluation of stem and leaf rust during the winter months. In comparison to other wheat production areas Makhathini has a relative temperate humid climate during the months from April to September (Meteoblue, 2012a). These conditions make Makhathini an ideal site for the inoculation and evaluation of stem and leaf rust since both thrives under these temperate humid conditions (Bolton *et al*., 2008; Leonard & Szabo, 2005; Pretorius *et al*., 2007).

Bethlehem on the other hand is located very high above sea level (1725 meters) and is situated far from the coast (350 kilometres). Due to it's topography Bethlehem has cold winter and spring time temperatures (Meteoblue, 2012b). Due to the early spring rains Bethlehem receives, a unique condition is created where low temperatures are accompanied by good humidity. This creates an ideal climate for the development and proliferation of stripe rust (Chen, 2005; Pretorius *et al*., 2007).

At both locations the MS-MARS lines along with the checks were planted in a randomized complete block design; with four replications. Two susceptible wheat lines, 'Morocco' and 'Rusty', were also planted to serve as spreaders. The disease evaluation trials had experimental plots which were 1.5 m in length and consisted of 2 rows with 40 cm inter-row spacing. Each line's overall reaction was taken by comparing each replication with each other and awarding the reaction which is more dominant over all the replications.

Table 3.7. Locations used in study for adult plant rust resistance evaluation.

Location	Location description	Rust evaluated			GPS coordinates
		Stem rust	Leaf rust	Stripe rust	
Bethlehem	Irrigation, marginal to high potential	-	-	X	28°11'11.1" S 28°13'00.3" E
Makhathini	Irrigation, High potential	X	X	-	27°24'08.7" S 32°11'00.6" E

To ensure optimum plant development at both Bethlehem and Makhathini, a total of 180 kg/ha nitrogen, using a fertilizer mixture of 6:3:1 (40), was applied. Standard agronomic practices were followed in applying this amount through the season. The irrigation schedule depended on the field water capacity of the soil. Regular soil moisture readings were taken on a weekly basis to keep the field water capacity on an acceptable level so that the plants did not go into any kind of water related stress.

At Bethlehem and Makhathini the inbred MS-MARS lines were field inoculated to evaluate each of the lines adult plant resistance to the three different rust pathogens. The technique used for field inoculation was the same method used by the University of the Free State which involved inoculation tents that ensures good infection conditions immediately after field inoculation (Z.A. Pretorius, personal communication, 2011). The inoculation was done by suspending the different pathotypes in Soltrol 170 (Chevron Phillips®, Woodlands, TX, USA), which is non-phytotoxic isoparafin oil. An ultra-low volume liquid dispersing sprayer was used to spray the spore suspension onto the spreaders and MS-MARS inbred lines. The inoculation took place in the late afternoon in order to shorten the time gap between spore placement on leaves and night time dew formation. To insure optimal infection, inoculation tents were erected on strategic laying spreader

plots to increase the probability of dew formation and to create a stable night time temperature. The inoculation tents were erected when sufficient time was given after inoculation to allow for evaporation of the Soltrol 170 to take place. The evaporation of the Soltrol 170 oil is important because if the oil is still present during dew formation, water drops would not be able to reach the spores and initiate spore germination (Roelfs *et al*., 1992). The inoculation tents were left over night for a minimum of 12 hours.

At Makhathini the stem rust pathotype UVPgt60, which is an Ug99 lineage race with virulence for both *Sr31* and *Sr24* (Pretorius *et al*., 2010) (Table 3.8); were inoculated as well as the leaf rust pathotype UVPrt13 (Table 3.9). This was done on two consecutive occasions, 70 and 80 days after planting. The first inoculation was done just before anthesis and the second just after anthesis. Adult plant infection types for leaf and stem rust of the MS-MARS inbred lines were recorded 44 days after the last inoculation. A second stem rust adult plant infection type was recorded 60 days after the last inoculation.

At Bethlehem the stripe rust pathotype 6E22A- (Table 3.10) were inoculated on the MS-MARS lines and the spreaders. This inoculation was done 40 days after planting when the wheat plants were still in it's vegetative phase. Adult plant infection types for 6E22A- were recorded 70 days after inoculation.

Adult plant disease response was rated as percentage infection, on a 0–100% scale.Whole plant reaction types where R = resistant, MR = moderately resistant, MS = moderately susceptible or S = susceptible as described by McIntosh *et al*. (1995).

Table 3.8. The avirulence/virulence formulae of the stem rust pathotype UVPgt60, which is an Ug99 lineage race with virulence for both *Sr31* and *Sr24*. The avirulence formulae indicating which *Sr*-genes still is effective in providing resistance to this virulent stem rust pathotype (Le Maitre, 2010; Pretorius *et al*., 2007).

Stem rust	Avirulence genes	Virulence genes
UVPgt60	*Sr13*, *Sr14*, *Sr21*, *Sr22*, *Sr25*, *Sr26*, *Sr27*, *Sr29*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr42*, *Sr43*, *Sr44*, *SrEm*, *SrTmp* and *SrSatu*	*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr16*, *Sr17*, *Sr24*, *Sr30*, *Sr31*, *Sr34*, *Sr38*, *Sr41* and *SrMcN*.

Table 3.9. The avirulence/virulence formulae of the two leaf rust pathotype UVPrt13. The avirulence formulae indicating which *Lr*-genes still is effective in providing resistance to this leaf rust pathotype (Le Maitre, 2010; Pretorius *et al*., 2007).

Leaf rust	Avirulence genes	Virulence genes
UVPrt13	*Lr3a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr16*, *Lr20* and *Lr30*	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr10*, *Lr14a*, *Lr15*, *Lr17*, *Lr24* and *Lr26*

Table 3.10. The avirulence/virulence formulae of the stripe rust pathotype 6E22A-. The avirulence formulae indicating which *Yr*-genes still is effective in providing resistance to this stripe rust pathotype (Le Maitre, 2010; Pretorius *et al*., 2007).

Stripe rust	Avirulence genes	Virulence genes
6E22A-	*Yr1*, *Yr3a*, *Yr4a*, *Yr4b*, *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr27*, *YrA*, *YrCle*, *YrCv*, *YrHVII*, *YrMor*, *YrSd*, *YrSp* and *YrSu*	*Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr17*and *Yr25*

4. Results and Discussion

4.1. Marker-assisted selection for the rust resistance genes

Sixty-four lines, sourced from the SU-PBLs pre-breeding MS-MARS population were screened for the three rust resistance gene complexes. A two-gene multiplex PCR was used to screen for *Sr24/Lr24* and *Lr37/Sr38/Yr17*. The 64 entries were also screened for the *Sr31/Lr26/Yr9* gene complex independently (Addendum A).

Frequencies were calculated for the presence of each of the genes in this subset of lines. The results were as follows: *Sr31/Lr26/Yr9* was present in 64% of the 64 lines. The *Lr37/Sr38/Yr17* complex found in 23% of the 64 lines screened while the *Sr24/Lr24* complex 77%. The complexes *Lr37/Sr38/Yr17* and *Sr24/Lr24* were associated with each other in 17 % of the 64 lines while *Lr37/Sr38/Yr17* and *Sr31/Lr26/Yr9* were found together in 9%. The largest percentage of the lines screened carried both the complexes *Sr24/Lr24* and *Sr31/Lr26/Yr9*; this pair was present together in 52% of the 64 MS-MARS lines screened. The lines carrying all three complexes represented 8% of the total 64 (Addendum B).

4.2. Double haploid production of selected MS-MARS lines, seed multiplication and final MAS of DH MS-MARS lines

The subset of 64 MS-MARS lines which amplified one or more of the three rust resistance gene complexes were incorporated into the SU-PBL's DH production programme to be made pure breeding. Out of the initial 64, 60 MS-MARS lines was included into the DH production programme, 15 were eventually selected based on seed availability and preliminary field screening during seed multiplication.

The 15 pure breeding MS-MARS lines were screened for all the markers listed in Table 3.2. The final genotype profile of each of them is depicted in Table 4.1. Although the markers for *Sr2* and *Lr34*/*Yr18* were not screened for in the initial subset of 64 MS-MARS lines, these genes were present in the pre-breeding MS-MARS population.

Table 4.1. The genetic profile of all 15 pure breeding MS-MARS lines. A dark cellin the molecular data columns represents a positive amplification while a "-"a non amplification.

Name	Genes screened through improved marker panel of the SU-PBL							
	Sr24/Lr24	***Sr38*-complex**	***Sr31*-complex**	***Lr34/Yr18***	***Sr2***	***Glu-Dy10***	***Glu-Dx5***	***Glu-Dy12***
SST056	■	■	-	■	-	■	■	-
SST047	■	■	-	-	-	■	■	-
SST806	-	-	-	-	-	-	-	■
SST867	-	-	-	-	-	■	■	-
US1010	-	-	-	-	-	-	-	■
MS-MARS-06	-	-	■	-	-	■	■	-
MS-MARS-07	■	-	■	■	-	-	-	■
MS-MARS-08	■	■	■	■	-	-	-	■
MS-MARS-09	■	-	-	■	-	■	■	-
MS-MARS-10	■	-	■	■	-	■	■	-
MS-MARS-11	■	■	-	■	-	-	-	■
MS-MARS-12	■	-	■	-	-	-	-	■
MS-MARS-13	■	-	■	-	-	■	■	-
MS-MARS-14	■	-	■	■	-	-	-	■
MS-MARS-15	■	■	-	■	-	-	-	■
MS-MARS-16	■	-	■	-	-	■	■	-
MS-MARS-17	■	-	-	■	-	■	■	-
MS-MARS-18	■	-	-	-	-	-	-	■
MS-MARS-19	■	-	-	-	-	■	■	-
MS-MARS-20	■	-	■	■	-	-	-	■

4.3. Field evaluation and final selection for crossing parents from MS-MARS lines

4.3.1. Data analysis and interpretation of the trial data

Each RCBD experiment was analysed using a GLM analysis as well as a NNA in Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada). This was done in order to determine whether to include adjusted (NNA) or unadjusted data into the final summarization table (Table 4.4) and AMMI analysis. The GLM analysis did not take any field trends into consideration while the NNA did. By using the adjacent residual method of the NNA (as available in Agrobase© Generation II) field trends have been taken into account and adjustments were made accordingly. The NNA adjusts the data for spatially correlated residuals, with the goal of increasing the overall precision of the experiment (Stroup & Mulitze, 1991).

At the three dryland locations, Langgewens, Welgevallen and Tygerhoek, small trends were identified by the NNA and the plot yields were adjusted accordingly (Addendum C & D). The efficiency of the NNA relative to the GLM is calculated through Agrobase© Generation II by the following equation; $1\text{-}SSE_a/SSE_u$, where SSE_a is the error sum of squares from the NNA ANOVA and SSE_u is error sum of squares from the GLM ANOVA (Yang *et al*., 2004). This equation calculates the percentage difference of residual explained from using the NNA instead of the GLM. Agrobase© Generation II also awards a NNA weight, which depicts the scale of field trends in each experiment by using the following equation; $1 - MSE_a/MSE_u$, where MSE_a is the error mean squares from the NNA ANOVA and MSE_u is error mean squares from the GLM ANOVA (Yang *et al*., 2004). When the NNA weight is above 0.1, a field trend is present. Values lower than 0.1 could indicate that field trends still persisted, however to a much smaller extent than values higher than 0.1 (Yang *et al*., 2004; D.K. Mulitze, personal communication, 2012).

All the descriptive statistics for each experiment is illustrated in Table 4.2.

By using the NNA over the GLM, residual variances were reduced by 4.34% at Langgewens, 7.07% at Welgevallen and by 11.26% at Tygerhoek. Agrobase© Generation II did not perform any nearest NNA on the data from Hartsvallei, this could most probably be due to trends which are too small and could not be estimated. Although the NNA weight Agrobase© Generation II calculated for Langgewens (0.030) was very small, it still carried out the adjustment of the data. For the other two experiments in the Western Cape the NNA weight calculated for Welgevallen and Tygerhoek

were 0.100 and 0.105 respectively which indicated that trends did exist. Although NNA weights were allocated for each of the three dryland experiments, the trends was not significant ($p > 0.05$).

The GLM model of Langgewens indicated that this experiment was the one with the lowest R^2value (58.6%). This indicated that the model used did not explain the variance in this experiment very well. By implementing the NNA, it is interesting to note that although the broad sense heritability (H^2)increased by 37.8%, the R^2 value dropped by 7.7% compared to the GLM. The NNA weight of 0.03 pointed at a very small field trend was present and due to this it adjusted the data accordingly. The NNA succeeded in reducing the residual variance (4.34%) and increasing in H^2 (37.8%). For this reason the NNA adjusted data from Langgewens was used in the summarized data table (Table 4.3) and the AMMI analysis. In order to visualize the trends at Langgewens each plot's residual were plotted using a grid layout of the experiment at Langgewens (Figure 4.1). Figure 4.1.a. shows the raw residual data from each plot in the experiment and figure 4.1.b. the NNA adjusted residual data. In figure 4.1.a. it was observed that there was a field trend in the experiment. In replications one and four, there was a trend in the plots to the higher residual values while at replication three, to the lower residual regions.

The R^2-value for Welgevallen (71.8%) was high indicating that the GLM model did well in explaining the majority of the variation (Table 4.2). As in the case with Langgewens, the NNA was also performed. The R^2 obtained from the NNA was 3.3% lower than that of the GLM, however the H^2 obtained from the NNA was higher, 86.5% vs. 62.4% from the GLM. The reduction in residual variance (7.07%) and the increase in H^2 by the NNA were the deciding factors in incorporating the adjusted NNA data in the summarized data table (Table 4.3) and the AMMI analysis. The plot residual grid of Welgevallen is included in Addendum E.

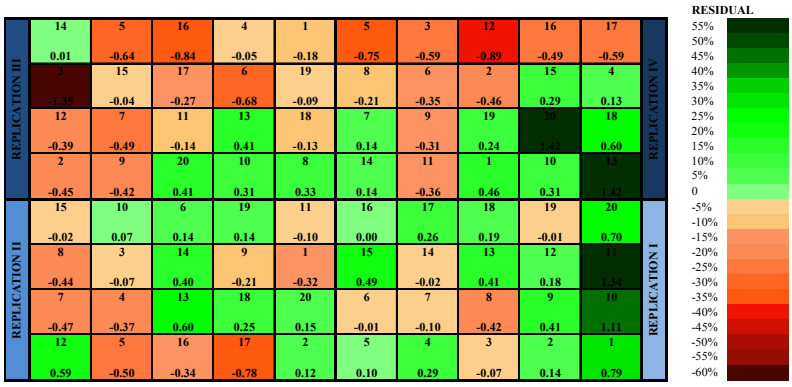
Although the R^2value obtained from the GLM at Tygerhoek was good (74.8%) small trends was picked up (NNA weight 0.105). By using the NNA at Tygerhoek the H^2 increased by 25.3% when compared to the GLM. The NNA succeeded in reducing the residual variance by 11.26%. The NNA adjusted data were chosen to represent the data for Tygerhoek in the summarized data table (Table 4.3) and the AMMI analysis. Tygerhoek's plot residual grid is included in Addendum E.

Since Agrobase© Generation II did not perform a NNA for Hartsvallei, only the unadjusted data was used in the summarized data table (Table 4.3) and the AMMI analysis. Hartsvallei's plot residual grid is included in Addendum E.

Table 4.2. Descriptive statistic data summary comparing the regular GLM's ANOVA statistics with the NNA's ANOVA statistics for each location.

	Langgewens		**Welgevallen**		**Tygerhoek**		**Hartsvallei**	
Parameter	**GLM**	**NNA**	**GLM**	**NNA**	**GLM**	**NNA**	**GLM**	**NNA**
Grand Mean (ton/ha)	2.40	2.39	1.41	1.41	5.62	5.62	7.86	-
R^2	58.6%	50.9%	71.8%	68.5%	74.8%	76.0%	84.6%	-
Residual SS	8.52	8.15	5.52	5.13	10.66	9.46	6.96	-
Residual MS	0.15	0.15	0.10	0.09	0.19	0.17	0.18	-
H^2	35.8%	73.6%	62.4%	86.5%	64.9%	90.2%	76.0%	-
C.V.	16.2%	15.9%	22.1%	21.4%	7.7%	7.3%	5.4%	-
NNA weight	-	0.030	-	0.100	-	0.105	-	-
NNA Efficiency	-	4.3%	-	7.1%	-	11.3%	-	-
L.S.D. (5%) (ton/ha)	0.46	0.45	0.37	0.36	0.51	0.49	0.59	-
Relative precision to GLM	-	100.1%	-	101.8%	-	110.1%	-	-

a.



b.

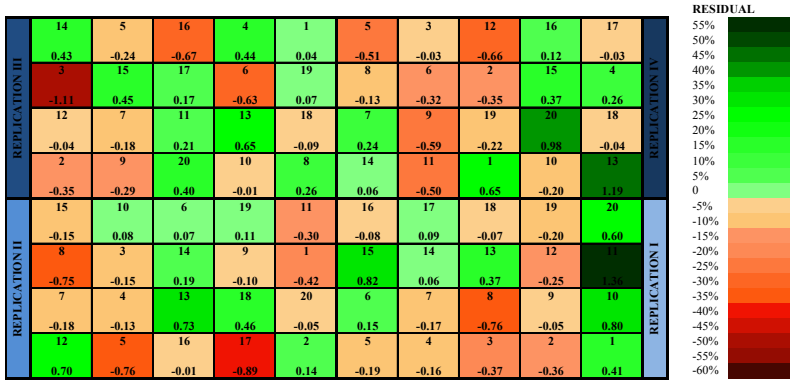


Figure 4.1. a). Unadjusted plot residuals, ascertained from the raw data, plotted on to the experimental layout of the Langgewens experiment. Note the clear trend from the bottom right corner to the top left corner of the experiment (NNA weight= 0.03). b). Adjusted plot residuals, ascertained from the adjusted NNA data, plotted on to the experimental layout of the Langgewens experiment. Note the elimination of the field trend when comparing the unadjusted residual grid layout with the NNA adjusted residual grid layout.

Table. 4.3. Summarized yield data of the trail consisting of four experiments planted at four locations in South Africa.

NAME	Langgewens			Welgevallen			Tygerhoek			Hartsvallei			Combined
	Yield (ton/ha)	Rank	% to check	Yield (ton/ha)	Rank	% to check	Yield (ton/ha)	Rank	% to check	Yield (ton/ha)	Rank	% to check	Average (ton/ha)
SST056	2.56	6	115	1.9	2	105	6.62	1	110	8.59	3	104	4.62
SST047	2.16	16	97	1.64	6	91	5.75	11	96	7.53	15	91	4.07
SST806	1.97	19	88	1.79	5	99	6.15	5	102	8.59	4	104	4.41
SST867	2.49	8	112	2.17	1	120	5.7	13	95	8.91	1	108	4.56
US 1010	1.97	20	88	1.56	7	86	5.95	7	99	7.67	13	93	4.03
MS-MARS-06	2.2	15	99	1.45	9	80	5.97	6	99	7.61	14	92	4.07
MS-MARS-07	2.32	12	104	1.46	8	81	5.51	15	92	8.48	6	103	4.21
MS-MARS-08	2.05	18	92	0.94	19	52	6.2	2	103	7.69	12	93	3.91
MS-MARS-09	2.14	17	96	1.04	17	57	4.67	18	78	8.78	2	106	3.88
MS-MARS-10	2.56	7	115	1.22	13	67	4.99	17	83	5.69	20	69	3.46
MS-MARS-11	2.58	4	116	1.18	14	65	6.17	3	103	8.26	9	100	4.28
MS-MARS-12	2.33	10	104	1.34	11	74	6.15	4	102	8.29	8	100	4.22
MS-MARS-13	3.12	1	140	1.9	3	105	5.87	8	98	6.81	18	82	4.23
MS-MARS-14	2.58	5	116	0.85	20	47	5.76	10	96	8.52	5	103	4.21
MS-MARS-15	2.76	3	124	1.83	4	101	5.74	12	96	8.41	7	102	4.46
MS-MARS-16	2.23	13	100	1.44	10	79	4.28	20	71	7.43	16	90	3.68
MS-MARS-17	2.22	14	100	1.11	15	61	5.82	9	97	8.00	10	97	4.09
MS-MARS-18	2.46	9	110	1.06	16	58	4.42	19	74	7.89	11	96	3.7
MS-MARS-19	2.33	11	104	1.29	12	71	5.11	16	85	7.30	17	88	3.77
MS-MARS-20	2.87	2	129	1.04	18	57	5.63	14	94	6.78	19	82	3.92
Grand Mean (ton/ha)	2.39			1.41			5.62			7.86			4.11
Check Mean (ton/ha)	2.23			1.81			6.01			8.26			4.35
Critical level above check mean (ton/ha)	2.68			2.17			6.49			8.85			4.58
Critical level below check mean (ton/ha)	1.78			1.45			5.52			7.67			4.13

By employing the AMMI analysis, the GEI were cpartitioned into three main IPCA axes. The first IPCA axis explained 59.3% and thus, according to Van Eeuwijk (1995) and Crossa *et al*., (1991) discriminated between the genotypes the best. The other two IPCA axes explained respectively 32.4% and 8.3% of the total GEI variance. Since the most GEI variance was explained by IPCA 1, it's scores together with the mean yields of each check and MS-MARS line were plotted onto an AMMI bi-plot (Van Eeuwijk, 1995; Crossa *et al*., 1991) (Figure 4.2). Due the high environmental variance and the low GEI variance, no environmental condition could be explained or described by the IPCA axes (Addendum F).

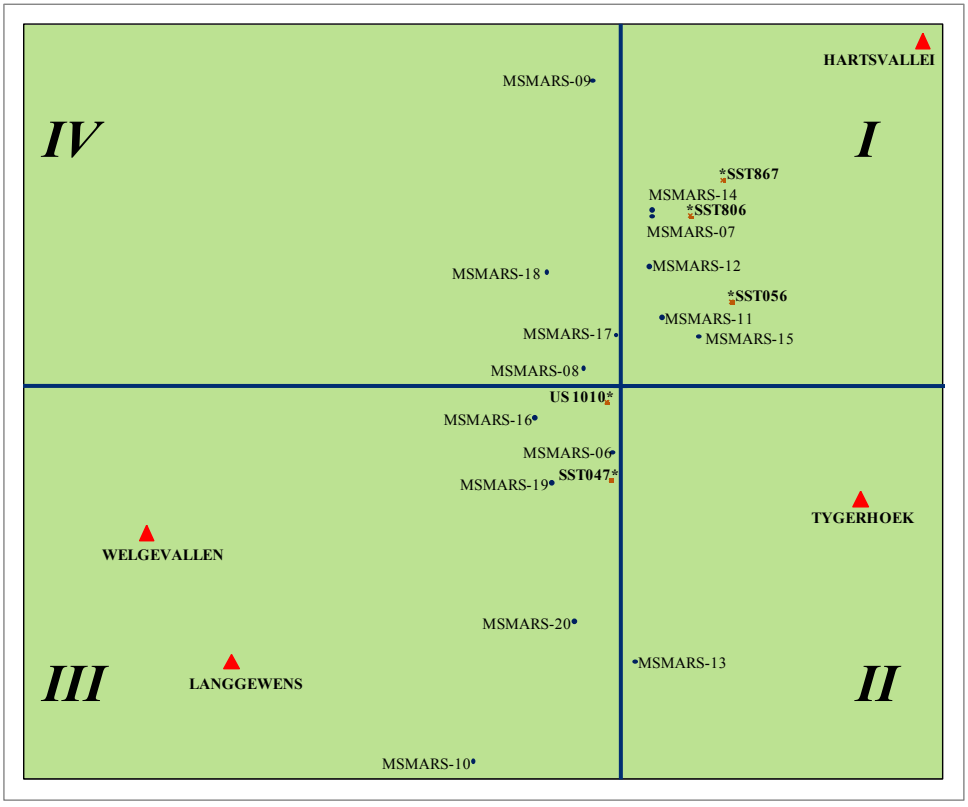


Figure 4.2. AMMI bi-plot. Plotting the IPCA 1 scores (y-axis) to the measured genotypic trial mean, expressed in ton/ha (x-axis), of each of the twenty lines and four experiments. The red triangles represent the four experiments conducted in the four environments and the blue dots the individual lines.

From the bi-plot the two mega-environments clearly could be distinguished from each other. Hartsvallei which represents the high potential irrigation mega environment is located very far from

the origin of the bi-plot. The three dryland environments of the Western Cape, which were the dryland mega-environment, all had negative IPCA 1 scores. Langgewens and Welgevallen were clustered together on the bi-plot. Such a clustering of locations could suggest that the majority of the lines responded the same at both these two locations. This is evident when considering Table 4.3. Tygerhoek being far to the right in the second quadrant was the dryland location with the best grand mean. The grouping of the dryland locations can further be explained by the fact that Langgegwens and Welgevallen represets the South Western Cape region while Tygerhoek the Rûens. The distribution of the four locations on the bi-plot is a very clear indication of the diverse environments each of them represent. The plotting of the locations on the bi-plot could also differ from year to year since each year could be different due to climatic influences. Genotypes clustering in close proximity to a location on the bi-plot are an indication that there is a relationship between the lines and that location; as is the case with the lines clustering near Hartsvallei ('SST867', 'SST806', MS-MARS-07, MS-MAR-14, MS-MARS-12, MS-MARS-11, MS-MARS-15 and 'SST056'). All the lines that are plotted in quadrants I and IV are well adapted to one of the two high potential locations either Hartsvallei or Tygerhoek. The lines plotted in quadrant I closest to the y-axis performed well at all four locations ('SST056', MS-MARS-15 & MS-MARS-11). The x-axis indicates the mean yield of all four experiments. Lines lying to the right where the x- and y-axis intercepts has yields beter that mean yield while those lying to the left lower that the mean yield. The closer a line is plotted to the y-axis, the more stable it is over all the locations (Van Eeuwijk, 1995; Crossa *et al*., 1991). This is the case with US1010 which showed stability at all four locations. MS-MARS-09 was plotted very far from the origin in quadrant IV which suggested that it was adapted very well to Hartsvallei which is high potential environment. This was the case when observing it's performance in Table 4.3 where it did poorly in the three dryland locations but performed very well at the high potential environment of Hartsvallei. The line MS-MARS-10 was clustered with MS-MARS-20. These two lines both had negative IPCA 1 scores plotted far from the origin which suggested that both were adapted more to lower potential environments. This was supported by Table 4.3 where both performed well at the two low potential sites, Langgewens and Welgevallen.

The AMMI and the information in Table 4.3 complimented each other in classifying each check and MS-MARS line for yield, adaptability and stability (Table 4.4). When consulting Table 4.4 it is possible to identify crossing parents for certain objectives in a breeding programme. When overall adaptability for both high and low potential environments is sought after in a breeding population,

lines from group 1 in Table 4.4 could be used. On the other hand when stability is more important, lines from group 6 could be used.

Table 4.4. Classification of each check and MS-MARS line according to yield, adaptability and stability.

Group	Name	Adaptability and stability characteristic
1	SST056 MS-MARS-11 MS-MARS-12 MS-MARS-15	Very well adapted to low and high potential mega environments in South Africa
2	SST867 SST806 MS-MARS-07 MS-MARS-14	Adapted to low and high potential environments in South Africa
3	MS-MARS-13	Very well adapted to the dryland high potential mega environments in South Africa
4	MS-MARS-09	Adapted only to irrigation high potential environments in South Africa
5	MS-MARS-17 MS-MARS-18	Adapted only to high potential environments in South Africa
6	MS-MARS-10 MS-MARS-20	Adapted to low potential environments in South Africa
7	US1010 MS-MARS-08 MS-MARS-16	Stable
8	SST047 MS-MARS-06 MS-MARS-19	Poor performing and not very well adapted or stable

4.3.2. Quality evaluation

Each characteristic had a certain tolerance level with which it could deviate from 'SST806'. In Addendum G the quality tables of each MS-MARS line is presented with their summarized quality data. Table 7.21 of Addendum G the quality characteristics of the quality standard, 'SST806', is summarized in. Tables 7.22 to 7.25 of Addendum G are those of the commercial checks compared to 'SST806'. Tables 7.26 to 7.40 of Addendum G are those of the MS-MARS lines compared to 'SST806'. Where the quality characteristics deviated more than the SAGL tolerance specifications, the deviation was indicated by a light blue colour. When the industrial quality characteristics (HLM, BFY and EX) were significantly higher than 'SST806's parameters, the positive deviations were indicated by light green. For industrial quality characteristics higher amounts of HLM, BFY and EX is of big importance for the milling and baking industry.

The high CV's calculated for each of the quality characteristics indicated the variability quality exerted over the four experiments. This emphasises the importance of using multi-locations in order to develop a concurrent quality profile for each specific genotype. When consulting Tables 7.21 through to 7.40, certain quality characteristics have lager CV's than others. This is because some quality characteristics is more influenced by genotype by environment interactions than others. For these the tolerance margins, from which they can deviate from, is larger than those which is not that notably effected by the environment.

The quality characteristics which were tested are all influenced both environmentally and genetically. Although the environment has a big effect on wheat quality, genetics also plays a very significant role in controlling wheat quality. The quality standard, 'SST806', is grown in all four experiments under the exact same environmental conditions the 15 MS-MARS lines were grown in. By comparing each of the MS-MARS line's quality to 'SST806', the environmental effect was minimized at each experiment and each of the line's quality was evaluated almost purely on grounds of it's genetic effect.

The MS-MARS lines which deviated within the tolerable margins set out by the SAGL, and which were regarded those having quality in line with 'SST806', were MS-MARS-15 and MS-MARS-19.

The MS-MARS lines which deviated with one quality characteristic were MS-MARS-08, MS-MARS-09, MS-MARS-18 and MS-MARS-20. MS-MARS-08 deviated on its bread loaf volume (VOL) where the test breads were 10% smaller than that of 'SST806'. Bread volume is a very important quality characteristic that wheat in South Africa needs to adhere to and therefore this line

will not be accepted as a bread wheat cultivar. MS-MARS-09 deviated on hectolitre mass (HLM), which is a very important physical characteristic for wheat. The average HLM of MS-MARS-09 over all four experiments was 4.9 kg/hl less than 'SST806'. This is outside the SAGL tolerance margin of 1.8kg/hl. MS-MARS-18 showed lower total flour extraction than 'SST806', thus this line will not make the cut either. On average, the total flour which was extracted from whole grains of MS-MARS-18, were 5.1% less than that of 'SST806'. The tolerable level is 1.5% lower than 'SST806'. MS-MARS-20 had lower HLM than 'SST806', although not as low as MS-MARS-09. MS-MARS-20's HLM were on average 2.7 kg/hl lower than 'SST806' making this line also not acceptible.

Although MS-MARS-13 deviated outside the tolerable margins for two quality characteristics, namely FN and ABS, it had a very good EX and PROT. Of the two characteristics, falling number (FN) and water absorption (ABS), MS-MARS-13 deviated from, FN is much more importance. The higher ABS could be seen as a positive since less flour from this line is necessary to make the same size bread loaf than one with a lower ABS (Miles, 2010). The FN is of concern because the low FN indicates that this line's seed has a bigger tendency to start germinating in the ears when exposed to prolonged rain after it is ripe than lines with higher FN. When seeds start to germinate the α-amylase breaks down the endosperm to make it accessible to be metabolised by the developing embryo. This influences the protein in the endosperm which ultimately reduces mixing tolerance of the dough (Miles, 2010).

Excluding MS-MARS-13, the rest of the MS-MARS lines all had two or more quality characteristic deviating outside the tolerable margins.

The quality evaluation worked very well in identifying the MS-MARS lines with the better quality. When compared to the commercial cultivars, 'SST056', 'SST047', 'SST867' and 'US1010' (Table 4.3.2.2); the variability in quality characteristics over all 15 MS-MARS lines was very evident. Since these lines originally came out of a yield and rust based RMS population, which was not subjected to a very aggressive quality screening programme, it could explain the wide array of quality characteristics observed.

The *Glu-1* quality scores of each of the cultivars and MS-MARS lines were calculated by using the scoring method of Payne *et al*., (1987). The *Glu-1* quality score of each commercial cultivar and MS-MARS line was calculated by adding the scores of the individual subunits it contains (Table

4.5). The HMW-GS, 5+10, encoded by genes on the D-genome, were awarded the highest score of 4. The HMW-GS, 7 and 6+8, encoded by genes on the B-genome, and the HMW-GS 4+12, encoded by genes on the D-genome, were awarded the lowest score of 1.

The HMW-GS which were determined for each of the cultivars and MS-MARS lines by means of the Agilent© 2100 Bioanalyzer Protein 230 LabChip Kit is illustrated in Table 4.6. The *Glu-1* scores which were calculated by the relationship of HMW-GS in each of the entries are illustrated in the last column (Table 4.6).

Table 4.5. Quality score assigned to individual or pairs of HMW-GS adapted from Payne *et al*.(1987).

	Chromosome		
Score	**1A**	**1B**	**1D**
4	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
3	-	14+15	-
2	-	7+9	2+12
2	-	-	3+12
1	null	7	4+12
1	-	6+8	-

To put these scores into perspective, it was compared to the physical quality of each of the MS-MARS lines (Table 4.7). The MS-MARS lines were grouped into four broad quality groups, namely above average, average, below average and poor quality based on the quality characteristics tested. The lines were allocated to these groupings by observing the number of deviations of quality characteristics each had when compared to the quality standard 'SST806' (Addendum G). The only exception was with MS-MARS-13 which had two deviations but was grouped in the average quality group which all had one deviation. The reason for grouping MS-MARS-13 with this group was due to it's two good industrial quality characteristics namely flour extraction (EX) and protein (PROT) which both were significantly better than 'SST806'.

The average *Glu-1* score for the *above average* quality group were 8.4. The average *Glu-1* scores for the *average* quality group dropped by 0.3 scoring units to 8.1. The *below average* quality

group's average *Glu-1* scores were 8.3 and the *poor* quality group 7.8. Although a small drop in average *Glu-1* score did exist between the quality groups, it might be difficult to implement the *Glu-1* score directly as a selection method since small differences in *Glu-1* score were perceived as big effects in quality and that HMW bands only explain 30-40% of wheat quality. This can be seen when looked at line MS-MARS-10 which deviated with three quality characteristics but still had the same *Glu-1* score as lines which were grouped in the *above average* and *average* quality group.

The three molecular markers that were used to screen for the HMW-GS 5, 10 and 12 correlated well with the HMW-GS 5, 10 and 12 scored through the Agilent© 2100 Bioanalyzer.

The quality evaluations worked well in identifying lines which could be used as parents for incorporating quality into a breeding population. All the checks and MS-MARS lines allocated to the *above average* and *average* quality groups could all be used as crossing parents for quality. However the ones in the *above average* group are more attractive to introduce quality (together with rust resistance) into a breeding population.

Table 4.6. The HMW-GS present in each of the lines and the corresponding calculated *Glu-1* score together with the HMW-GS markers screened for. A dark cellin the molecular data columns represents a positive amplification while a "-"a non amplification.

	***Glu*- subunits**				***Glu*-markers**		
Name	**1A**	**1B**	**1D**	***Glu-1* Score**	***Glu-Dy10***	***Glu-Dx5***	***Glu-Dy12***
SST 056	2*	7+8	2+12	8			-
SST 047	2*	7	5+10	6			-
SST 806	2*	7+8	2+12	8	-		-
SST 867	2*	7+8	5+10	10			-
US 1010	null	7+8	2+12	6	-	-	
MS-MARS-06	1	7+9	5+10	9			-
MS-MARS-07	1	7	2+12	6	-	-	
MS-MARS-08	2*	14+15	2+12	8	-	-	
MS-MARS-09	2*	17+18	5+10	10			-
MS-MARS-10	2*	7+9	5+10	9			-
MS-MARS-11	1	14+15	2+12	8	-	-	
MS-MARS-12	1	14+15	2+12	8	-	-	
MS-MARS-13	2*	7+9	5+10	9			-
MS-MARS-14	1	14+15	2+12	8	-	-	
MS-MARS-15	1	14+15	2+12	8	-	-	
MS-MARS-16	2*	7	5+10	8			-
MS-MARS-17	2*	7	5+10	8			-
MS-MARS-18	2*	14+15	2+12	8	-	-	
MS-MARS-19	1	7+8	5+10	10			-
MS-MARS-20	1	14+15	2+12	8	-	-	

4.3.3. Field rust inoculation and adult plant resistance evaluation

The technique of using inoculation tents to create an initial high humid night environment on strategic positioned spreaders, worked very well in achieving disease establishment. This humid night micro climate, created by the inoculation tents, was more evident at Bethlehem than at Makhathini (Figures 4.3 & 4.4). At Bethlehem, when the stripe rust inoculation was done, natural night time dew formation was hampered due to dry late autumn winds. Since natural dew formation was not as prominent at Bethlehem as at Makhathini, the spreaders on which the inoculation tents were erected on through the night had very good dew formation on their leaves. No dew formation was observed on the leaves of the spreaders which had no inoculation tents erected over them. Dew and low temperature is very important for stripe rust spore germination and infection. After inoculation there was a clear difference between spreaders which did not have the inoculation tents erected over them and those which had the inoculation tents over them at Bethlehem. After inoculation the irrigation schedule were manipulated to create an artificial high humid environment during the night by scheduling the irrigation between six o'clock and eight o'clock at night. This assisted in further infection resulting from rust spores which came from developing uredinia on spreaders infected by means of the inoculation tent technique.

Figure 4.3. Inoculation tent at Bethlehem on the left (note the early stage of the wheat's development) and the presence of water droplets on the leaves of the spreaders covered with the mini-tunnels, on the right, indicating a humid mini-environment.

Figure 4.4. Placement of inoculation tents at Makhathini on the left, and the effective dew formation on the right.

At Makhathini no special irrigation scheduling needed to be done because natural dew formation happened every night and the dew stayed on the leaves until nine o'clock each morning. This was very conductive for continues stem and leaf rust infection.

The individual stem, leaf and stripe rust infection types taken at adult plant stage, over the 2011 and 2012 cycles, are shown in Addendum H. Due to sub-optimal rust infections recorded during 2011 at Makhatini, only the leaf and stem rust reactions recorded in 2012 was used to classify each check and MS-MARS line's effective resistance to leaf and stem rust. In Table 4.9 the effective adult plant infection types along with the corresponding molecular marker data are summarized in. All the checks and MS-MARS lines were grouped into 9 groups according to each molecular marker profile of rust resistance genes and complexes.

Table 4.8. Overall field rust reaction and molecular marker data of each entry. A dark cellin the molecular data columns represents a positive amplification while a "-"a non amplification.

		Effective adult plant resistance			Marker profile of rust resistance genes and complexes				
Group	Name	YR	SR	LR	*Sr2*	*Lr34/Yr18*	*Sr31/Lr26/Yr9*	*Sr24/Lr24*	*Sr38/Lr37/Yr17*
1	SST047	R	MS	MS	-	-	-		
2	SST806	S	S	MS	-	-	-	-	-
	SST867	S	S	R	-	-	-	-	-
	US1010	-	R	MR	-	-	-	-	-
3	MS-MARS-06	S	S	S	-	-		-	-
4	MS-MARS-08	MR	MR	MS	-				
5	MS-MARS-07	R	R	MS	-				-
	MS-MARS-10	MR	MS	MS	-				-
	MS-MARS-14	R	MS	R	-				-
	MS-MARS-20	S	MS	R	-				-
6	MS-MARS-09	R	MR	S	-		-		-
	MS-MARS-17	MS	S	MS	-		-		-
7	SST056	R	MR	S	-		-		
	MS-MARS-11	MS	MS	MS	-		-		
	MS-MARS-15	R	R	MS	-		-		
8	MS-MARS-12	MR	MS	R	-	-			-
	MS-MARS-13	MS	MS	R	-	-			-
	MS-MARS-16	MS	R	S	-	-			-
9	MS-MARS-18	MS	MS	R	-	-	-		-
	MS-MARS-19	S	S	MS	-	-	-		-

The avirulence / virulence formula for the stem rust pathotype UVPgt60, inoculated at Makhathini, is shown in Table 3.8. According to this formula, none of the stem rust resistance genes that were traced by the panel of markers is truly effective for UVPgt60, which is an Ug99 lineage race. The durable stem rust resistance gene *Sr2* only in conjunction with other effective stem rust resistance genes can provide sufficient protection against this pathotype (Pretorius *et al*., 2010).

The avirulence / virulence formula for the leaf rust pathotype UVPrt13, inoculated at Makhathini, is shown in Table 3.9. From the markers screened by the marker panel, none according to the avirulence / virulence formula is effective against this pathotype. Although the durable leaf rust resistance gene *Lr34* can provide resistance, it is only when other effective genes also is present (Singh *et al*. 2005).

The avirulence / virulence formula for the stripe rust pathotype 6E22A-, inoculated at Bethlehem, is shown in Table 3.10. From the markers screened by the marker panel only *Yr9*, according to the avirulence / virulence formula, is effective against this pathotype. Although the durable stripe rust resistance gene *Yr18* can provide resistance, it is only when other effective genes also is present (Singh *et al*. 2005).

Group 1 consisted of 'SST047' which had a unique marker profile. It carries the two rust resistance gene complexes *Sr24*/*Lr24* and *Sr38*/*Lr37*/*Yr17*. Since 'SST047' gave a moderate susceptible adult plant reaction to UVPgt60, it is suggested that there was no other background genes in this check. The leaf rust resistance genes *Lr24* and *Lr37* is both ineffective against UVPrt13 suggesting that 'SST047' carries minor resistance genes for leaf rust. 'SST047' gave a resistance reaction for the stripe rust pathotype 6E22A-. The marker panel only detected the stripe rust resistance gene *Yr17* which is not effective against 6E22A-. It is thus suggested that 'SST047' carries additional minor stripe rust resistance genes in it's background.

Group 2 consisted of 'SST867', 'SST806' and 'US1010'. The marker profile of these three checks showed that none of the screened genes was present. The two checks 'SST806' and 'SST867' showed no resistance to the stem rust pathotype UVPgt60 while 'US1010' did. This suggested that 'US1010' carries other additional minor stem rust resistance genes which contributed to it's good resistance. 'SST867' and 'US1010' were both resistant to the leaf rust pathotypes UVPrt13 suggesting both carry additional minor resistance genes for leaf rust. 'SST806' gave a moderate

susceptible reactions to the leaf rust which also could indicate the presence of minor resistance genes in it's background since a total susceptible reaction was not recorded. Both 'SST806' and 'SST867' were very susceptible to the stripe rust pathotype 6E22A- indicating that these two has no stripe rust resistance genes in the background. In this group, 'US1010' was the more resistant one suggesting that this check carries very effective minor resistance genes.

Group 3 consisted of MS-MARS-06 which had a unique marker profile in carrying only the rust resistance gene complexes *Sr31*/*Lr26*/*Yr9*. It's adult plant resistance to all three rust pathogens was not good. MS-MARS-06 carries no resistance genes for UVPgt60 in it's background. This was shown by the total susceptibility it had for UVPgt60 in it's adult plant stage. The leaf rust resistance gene that accompanied *Sr31* was *Lr26* which did not provide any protection against UVPrt13. It is interesting to note the adult plant reaction MS-MARS-06 showed against 6E22A-. Although 6E22A- is avirulent for the stripe rust resistance gene *Yr9*, it still succeeded in infecting MS-MARS-06.

Group 4 consisted MS-MARS-08 which carries the most genes, when compared to the rest, screened by the panel of markers. The markers amplified in this line were *Lr34/Yr18*, *Sr31/Lr26/Yr9*, *Sr24/Lr24* and *Sr38/Lr37/Yr17*. MS-MARS-08 had good resistance against the stem rust pathotype UVPgt60 as well as the stripe rust pathotype 6E22A-. It was only for UVPrt13 for which MS-MARS-08 was not resistant to. The amplified stem rust resistance genes present in this line is not effective against UVPgt60 suggesting that other additive minor stem rust resistance genes also are present. The stripe rust resistance genes that was amplified were *Yr9*, *Yr17* and *Yr18* of which *Yr9* and *Yr18* both is effective against 6E22A-. The only leaf rust resistance gene effective against UVPrt13, in this line, is the durable resistance gene *Lr38*, which gave the line a moderate susceptible reaction.

Group 5 consisted of MS-MARS-07, MS-MARS-10, MS-MARS-14 and MS-MARS-20. These lines all carry the rust resistance gene complexes *Lr34/Yr18*, *Sr31/Lr26/Yr9* and *Sr24/Lr24*. The line having the best adult plant resistance to UVPgt60 was MS-MARS-07. The stem rust resistance genes present in these lines is not effective against UVPgt60. This indicates that MS-MARS-07 carries additive minor resistance genes. Only MS-MARS-14 and MS-MARS-20 had good resistance to UVPrt13 suggesting that both, in addition with *Lr34*, carry other additional minor leaf rust resistance. The other two leaf rust resistance genes, *Lr26* and *Lr24*,are not effective against UVPrt13. The majority of the MS-MARS lines in this group were resistant to 6E22A- except MS-

MARS-20. This is interesting since all of them carry the stripe rust resistance genes *Yr9* and *Yr18* that is effective against 6E22A- (Pretorius *et al*., 2007; Le Maitre, 2010).

Group 6 consisted of MS-MARS-09 and MS-MARS-17. Both these lines all carried the rust resistance gene complexes *Lr34/Yr18* and *Sr24/Lr24*. MS-MARS-09 had good resistance to UVPgt60 while MS-MARS-17 did not. Since only *Sr24* was detected, suggests that MS-MARS-09 carry additional minor stem rust genes. These additional minor genes are not present in MS-MARS-17 since it had a susceptible adult plant reaction to UVPgt60. Both these lines was susceptible to UVPrt13, however MS-MARS-09 was more susceptible than MS-MARS-17. This indicated that MS-MARS-17, in addition with *Lr34*, also carry additional minor genes. MS-MARS-09 had very good resistance against 6E22A- which suggest it carries additional minor stripe rust resistance genes. The moderate susceptible reaction MS-MARS-17 had could indicate that only *Yr18* was present.

Group 7 consisted of the check, 'SST056' and the two MS-MARS lines, MS-MARS-11 and MS-MARS-15. All three carries the rust resistance gene complexes *Lr34/Yr18*, *Sr24/Lr24* and *Sr38/Lr37/Yr17*. SST056 and MS-MARS-15 both were resistant to UVPgt60. The two stem rust resistance genes which were detected are not effective against UVPgt60 indicating that both 'SST056' and MS-MARS-15 carries additional minor stem rust resistance genes. These additional minor genes are not present in MS-MARS-11 since it was susceptible to UVPgt60. 'SST056' was the most susceptible for the UVPrt13, while MS-MARS-11 and MS-MARS-15 was moderately susceptible. Since all three has the same marker profile, it is suggested that MS-MARS-11 and MS-MARS-15 carries additional minor genes which together with *Lr34* gave the moderate susceptible reactions against UVPrt13. 'SST056' and MS-MARS-15 was resistant to 6E22A- while MS-MARS-11 not. Due to the same stripe rust marker profile these three has, MS-MARS-11 most probably do not carry the additional minor resistance genes which 'SST056' and MS-MARS-15 carries.

Group 8 consisted of MS-MARS-12, MS-MARS-13 and MS-MARS-16. These lines all carry the two rust resistance gene complexes *Sr31/Lr26/Yr9* and *Sr24/Lr24*. Only MS-MARS-16 was resistant to UVPgt60. Both *Sr31* and *Sr24* is ineffective for UVPgt60 which indicates that MS-MARS-16 carries additional minor stem rust resistance genes in it's background. Although MS-MARS-16 carries additional minor stem rust resistance genes, it is not the same for leaf rust. MS-MARS-16 was susceptible for UVPrt13 while MS-MARS-12 and MS-MARS-13 was not. UVPrt13

has virulence for both *Lr26* and *Lr24* which suggests that MS-MARS-12 and MS-MARS-13 carry additional minor leaf rust resistance genes. Although the stripe rust resistance gene *Yr9* is carried by all three lines in this group, it is interesting to note that only MS-MARS-12 was resistant to 6E22A.

Group 9 consisted of MS-MARS-18 and MS-MARS-19. Both these lines carry the rust resistance gene complex *Sr24/Lr24*. Both these two lines were susceptible for UVPgt60; however MS-MARS-19 more so than MS-MARS-18. The difference in adult plant resistance reactions for UVPgt60 could be because MS-MARS-18 carries additional minor stem rust resistance genes with very small effects which produced moderate susceptible stem rust reactions. The only leaf rust resistance gene traced in these two lines was *Lr24*. Although UVPrt13 is virulent for *Lr24*, MS-MARS-18 still had very good resistance against this pathotype. This good resistance could be ascribed to the presence of very effective additional minor leaf rust resistance genes in it's background. No stripe rust resistance genes were found by the marker panel in these two lines explaining the susceptible reactions both had against 6E22A-. However, MS-MARS-18's adult plant reaction was not as susceptible as that of MS-MARS-19 indicating the presence of additional minor stripe rust resistance genes in this line.

The rust pathotypes used in this study were virulent for the majority of the genes that was screened for in this material. Keeping this in mind, it must be presumed that the resistance to moderate susceptible reactions in some of the checks and MS-MARS lines effectively came from unknown additional minor genes. Minor genes are seen by wheat breeders and pathologists to go hand-in-hand with durable resistance (Singh *et al*., 2011; Pretorius *et al*., 2010; Parlevliet, 1995; Parlevliet, 1993).

With the knowledge of each entry's rust resistance genes screened for by the panel of markers and each adult plant rust reactions (Table 4.8), the minor gene status of each was determined (Table 4.9).

Table 4.9. Determined minor resistance genes carried by each check and MS-MARS line.

Name	Minor genes carried
SST 056	*Stem and stripe rust*
SST 047	*Leaf and stripe rust*
SST 806	*Leaf rust*
SST 867	*Leaf rust*
US 1010	*Stem and leaf rust*
MS-MARS-06	*None*
MS-MARS-07	*Stem and leaf rust*
MS-MARS-08	*Stem rust*
MS-MARS-09	*Stem and leaf rust*
MS-MARS-10	*Leaf rust*
MS-MARS-11	*Leaf rust*
MS-MARS-12	*Leaf rust*
MS-MARS-13	*Leaf rust*
MS-MARS-14	*Leaf rust*
MS-MARS-15	*Stem, leaf and stripe rust*
MS-MARS-16	*Stem rust*
MS-MARS-17	*Leaf rust*
MS-MARS-18	*Stem, leaf and stripe rust*
MS-MARS-19	*Leaf rust*
MS-MARS-20	*Leaf rust*

With major rust resistance genes becoming ever more vulnerable to a constantly changing rust pathogen, durable rust resistance gets increasingly more important (Singh *et al*., 2011). Since determining each entry's minor gene status, these entries can be chosen as crossing parents to introduce durable resistance into a breeding population for each of the three rust pathogens. When choosing minor resistant gene carrying parents from Table 4.9, it would be wise choosing checks or MS-MARS lines which carry minor genes of resistance for more than one of the rust pathogens. One of the main purposes of the MS-MARS breeding scheme is to increase the frequencies of minor resistance genes in a wheat population (Marais & Botes, 2009). By reincorporating lines identified in this study with minor genes of all three rust pathogens in the existing MS-MARS pre-breeding population, can facilitate in accumulating minor resistance genes. The two MS-MARS lines best to introduce minor resistance genes from the 15 entries are MS-MARS-15 and MS-MARS-18 (Table 4.9).

5. Conclusion

The markers used for screening the complexes *Sr24/Lr24*, *Lr37/Sr38/Yr17* and *Sr31/Lr26/Yr9* in the 64 MS-MARS lines performed well in identifying those lines which carries these resistance complexes. From the initial subset of 64 lines, 60 were chosen to advance to the DH phase and seed multiplication. The 60 lines either carried one or more of the three rust resistance complexes. The genes that were the most prominent were *Sr31*/*Lr26*/*Yr9* and *Lr24*/*Sr24*. The selected lines were incorporated into a DH seed multiplication phase. After 4 cycles of seed increases and preliminary field evaluation during multiplication, 15 lines were chosen and subjected to multi-location evaluations. The trials were done to evaluate general yield adaptability and stability, bread making quality and rust resistance.

From a breeding perspective, a superior genotype needs adaptability and/or stability. This is especially important when a breeding programme is focused on breeding for superior performing resistant varieties. Since most sources of resistant genotypes comes from backgrounds poorly adapted to South African conditions, it is necessary to cross these sources with good adaptive and/or stable genotypes. The extensive multi-location yield evaluation carried out in this study between the dryland and the irrigated mega environments of South Africa aided in identifying genotypes from the 15 MS-MARS lines with good adaptability and stability. By using such lines as parents in a pre-breeding effort to incorporate resistance from an exotic source, the chance of selecting progeny with resistance and adaptability or stability is increased.

Since the MS-MARS population developed by Marais & Botes (2003) did not undergo stringent quality evaluation during it's development, considerable quality variation was observed within the 15 MS-MARS lines. Nonetheless, good quality lines were still identified in the MS-MARS group which could be used as parents contributing quality characteristics to a breeding population. The molecular markers for the genes encoding the HMW-GS 5, 10 and 12 correlated well with HMW-GS subunits observed with the Agilent© 2100 Bioanalyzer. This is a positive finding since molecular markers is much more effective in identifying individual bands than evaluating and scoring the bands on SDS-PAGE. Scoring the bands on SDS-PAGE needs a very trained eye to distinguish between the different bands allocated to the different subunits.

The multi-location adult plant rust resistance evaluation made it possible to identify which of the checks and MS-MARS lines carried minor genes for resistance to the three rust pathogens. The rust pathotypes inoculated at the sites where the rust evaluation was done, was very virulent according

to each avirulence/virulence formulae. Since the majority of the rust resistance genes identified in the checks and MS-MARS lines were not effective for these pathotypes, the presumption was made that resistance came from additional minor genes in the background. By frequently using genotypes carrying minor resistance genes as crossing parents one can enrich breeding populations with effective durable resistance against wheat diseases.

The lines which performed the best in each of the traits evaluated for are listed in Table 5.1.

Table 5.1. Lines performing the best for each characteristic evaluated.

Evaluation	**Specific characteristic**	**MS-MARS lines**
General yield adaptability and stability	Low and high potential	MS-MARS-07, MS-MARS-11, MS-MARS-12, MS-MARS-14 & MS-MARS-15
	Low potential	MS-MARS-13
Bread quality	Above average	MS-MARS-15 & MS-MARS-19
	Average	MS-MARS-08, MS-MARS-09, MS-MARS-13, MS-MARS-18 & MS-MARS-20
Rust resistance	Minor genes for the three rust pathogens	MS-MARS-15 & MS-MARS-18
	Minor genes for two rust pathogens	MS-MARS-07 & MS-MARS-09

The aims and objectives of this project were achieved. From the initial 64 lines, 15 MS-MARS lines were incorporated into the multi-environment evaluations. From these 15 lines, individuals were identified having superior traits for all three characteristics tested for.

In future studies the lines which performed the best could the re-incorporated into the existing MS-MARS prebreeding programme of the SU-PBL. The frequencies of desired alleles could be increased in this manner. Since the majority of these characteristics are influenced by quantitatively inherited alleles, reimplementing these lines through RMS could increase the frequencies of these alleles in the existing SU-PBL prebreeding population.

Given the access to technologies in mapping populations for quantitatively inherited traits, the MS-MARS lines identified in this study carrying minor resistance genes can be typed. In doing this the minor genes can be traced and can facilitate incorporating them into other populations.

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7. Addendum

7.1. Addendum A: Gel photos and molecular data of the 64 MS-MARS lines sourced from SU-PBL.

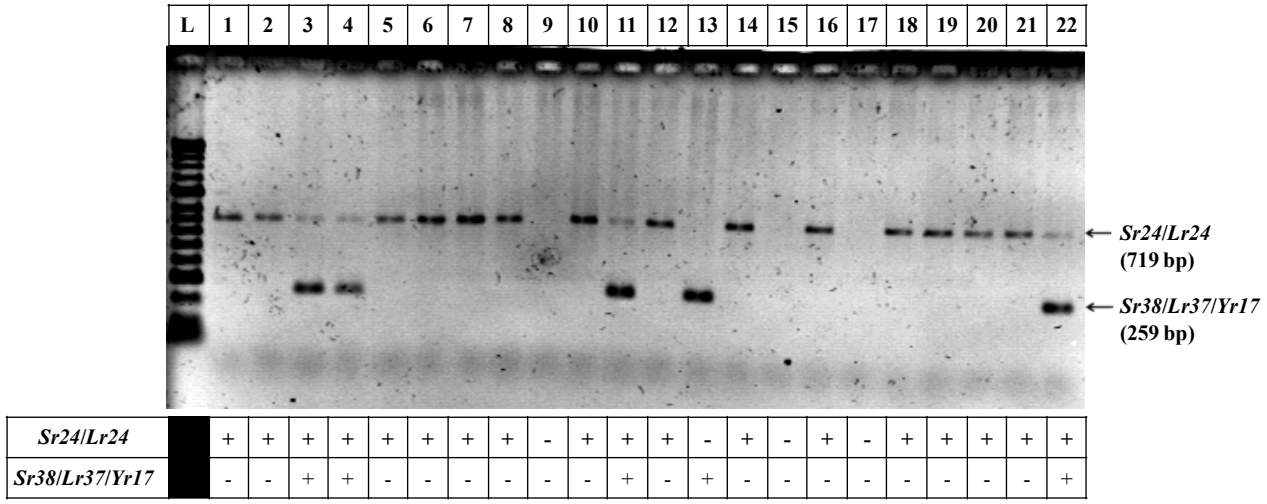
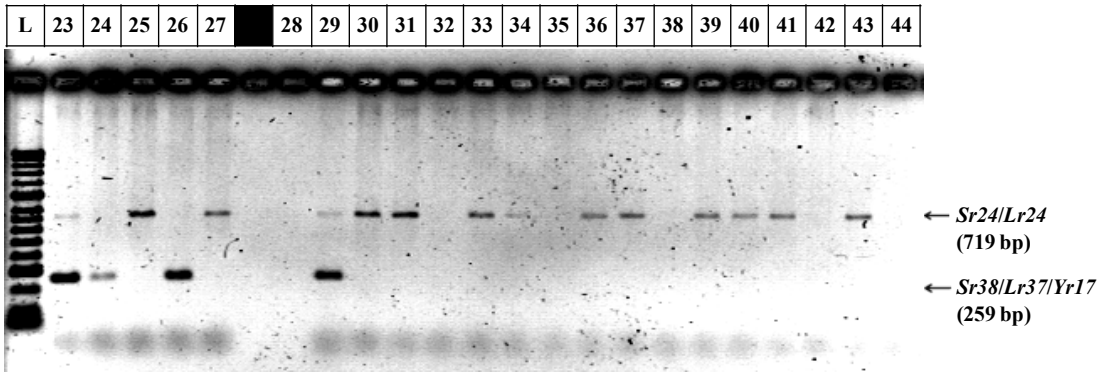
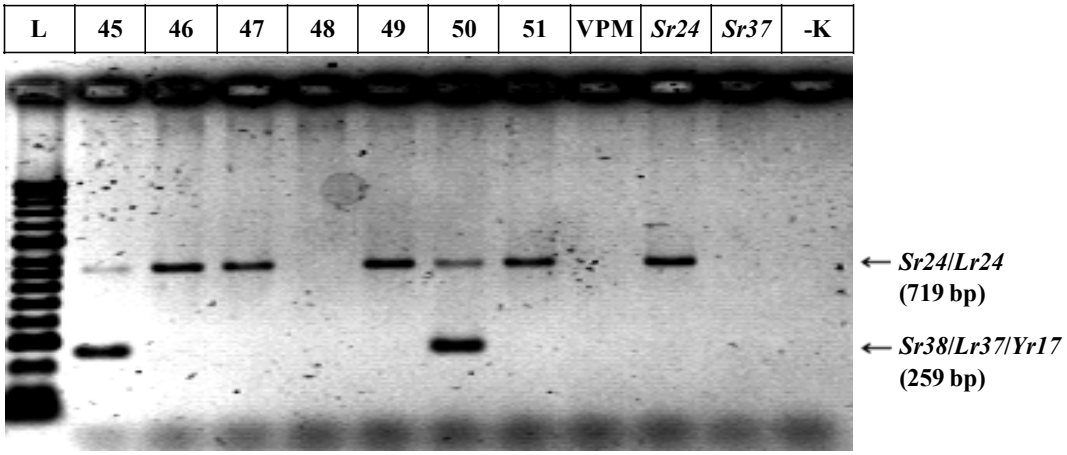


Figure 7.1. Gel photo showing different sized fragments of the *Sr24/Lr24* and *Lr37/Sr38/Yr17* multiplex PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 1 – 23: DNA samples of entries 1 to 22 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.



Sr24/Lr24		+	-	+	-	+			+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-
Sr38/Lr37/Yr17		+	+	-	+	-			+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 7.2. Gel photo showing different sized fragments of the *Sr24/Lr24* and *Lr37/Sr38/Yr17* multiplex PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 24: DNA samples of entries 23 to 44 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.



Sr24/Lr24		+	+	+	-	+	+	+	-	+	-	-
Sr38/Lr37/Yr17		+	-	-	-	-	+	-	-	-	-	-

Figure 7.3. Gel photo showing different sized fragments of the *Sr24/Lr24* and *Lr37/Sr38/Yr17* multiplex PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 8: DNA samples of entries 45 to 51 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.Lane 9-VPM, lane 10-*Sr24* positive, lane 11-*Sr37* positive and lane 12-negative control.

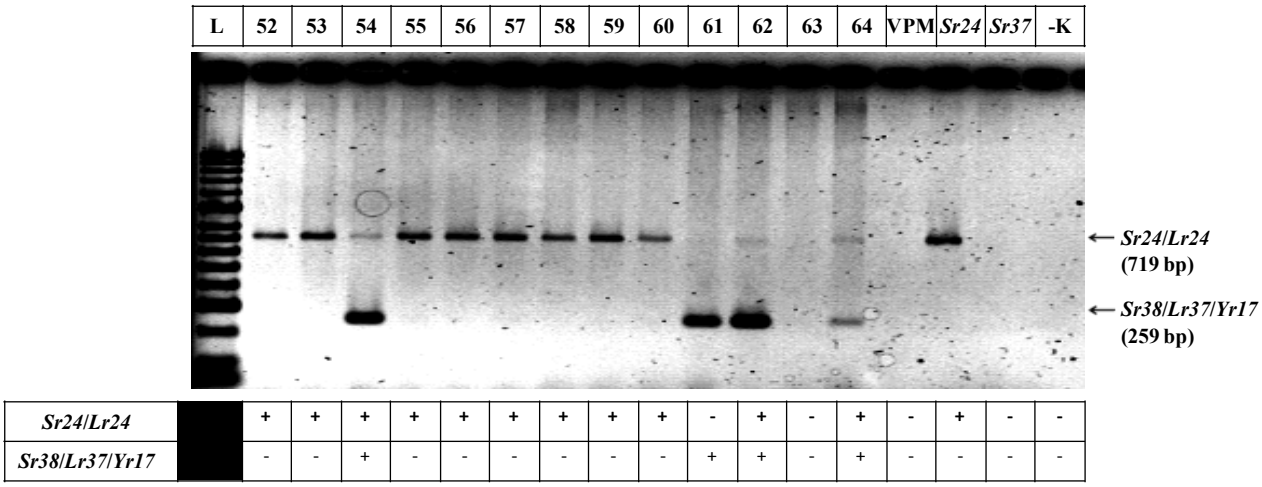


Figure 7.4. Gel photo showing different sized fragments of the *Sr24/Lr24* and *Lr37/Sr38/Yr17* multiplex PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 14: DNA samples of entries 52 to 64 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.Lane 15-VPM, lane 16-*Sr24* positive, lane 17-*Sr37* positive and lane 18-negative control.

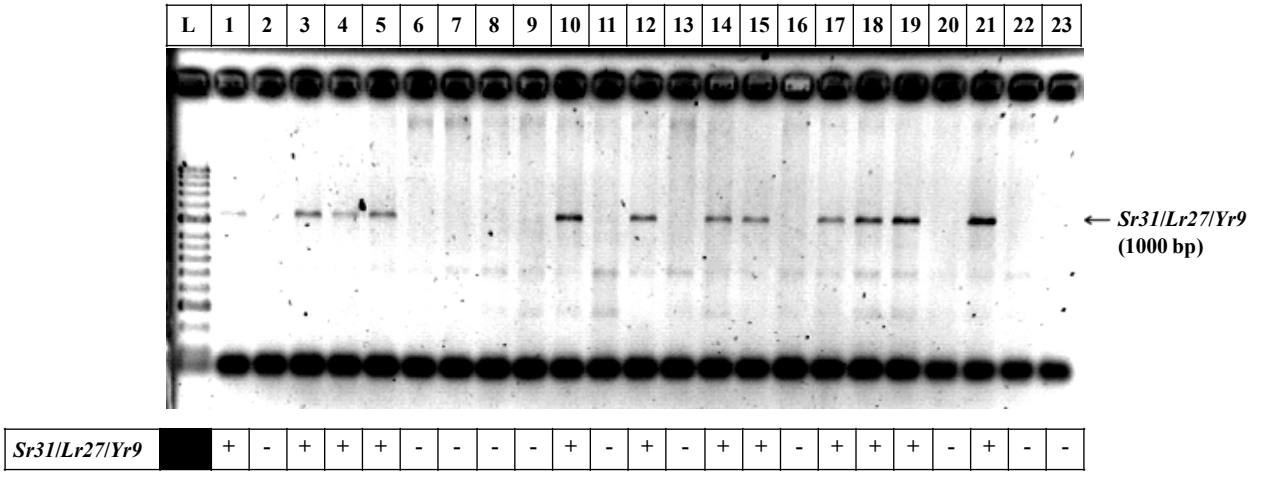


Figure 7.5. Gel photo showing fragments of the *Sr31/Lr26/Yr17* PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 24: DNA samples of entries 1 to 23 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.

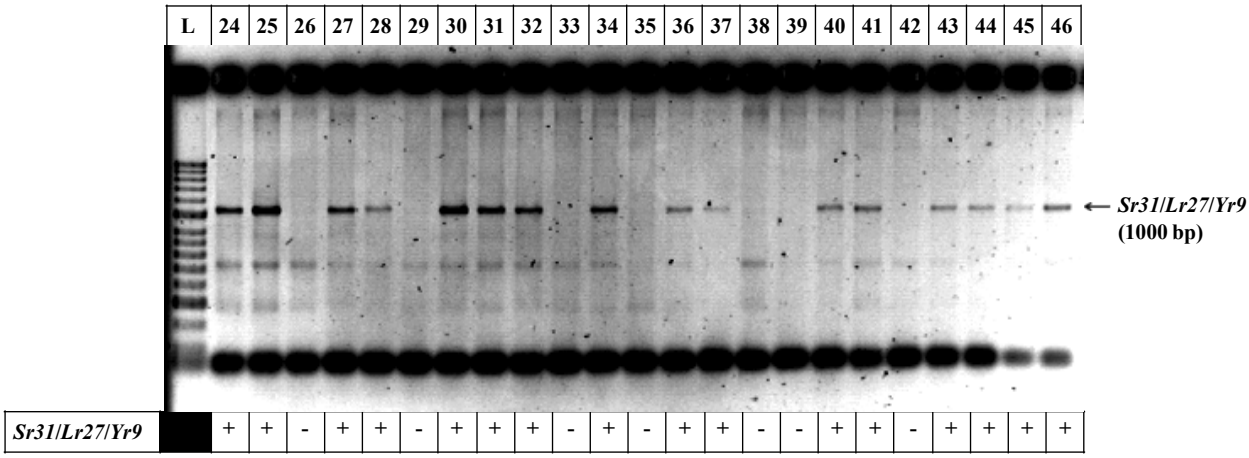


Figure 7.6. Gel photo showing fragments of the *Sr31/Lr26/Yr17* PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 24: DNA samples of entries 24 to 46 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL.

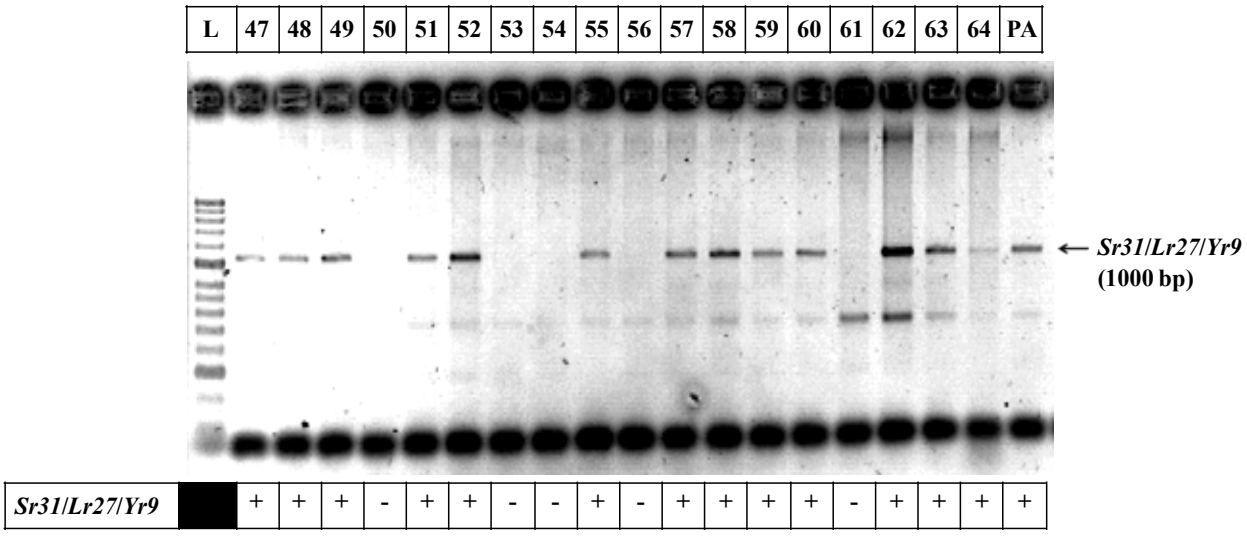


Figure 7.7. Gel photo showing fragments of the *Sr31/Lr26/Yr17* PCR (2% agarose gel elecrophorized for 100min at 120V). Lane I: Promega® - 100bp DNA Ladder. Lanes 2 – 19: DNA samples of entries 47 to 64 of the subset of 64 MS-MARS lines from the MS-MARS pre-breeding program of SU-PBL. Lane 20-Pavon (positive control).

7.2. Addendum B: Molecular data summarized of the 64 MS-MARS lines sourced from MS-MARS pre-breeding program of SU-PBL.

Table 7.1. Molecular data summary for the 64 MS-MARS lines sourced from MS-MARS pre-breeding program of SU-PBL.A dark cellin the molecular data columns represents a positive amplification while a "-"a non amplification.

MS-MARS	*Lr24*/*Sr24*	*Sr38*/*Lr37*/*Yr17*	*Sr31*/*Lr26*/*Yr9*	*Sr24*-complex &*Sr31*-complex	*Sr24*-complex &*Sr38*-complex	*Sr31*-complex &*Sr38*-complex	*Sr24*-complex, *Sr38*-complex &*Sr31*-complex	None
1		-			-	-	-	-
2		-	-	-	-	-	-	-
3								-
4								-
5		-			-	-	-	-
6		-	-	-	-	-	-	-
7		-	-	-	-	-	-	-
8		-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	
10		-			-	-	-	-
11			-	-		-	-	-
12		-			-	-	-	-
13	-		-	-	-	-	-	-
14		-			-	-	-	-
15	-	-		-	-	-	-	-
16		-	-	-	-	-	-	-
17	-	-		-	-	-	-	-
18		-			-	-	-	-
19		-			-	-	-	-
20		-	-	-	-	-	-	-

Table 7.1. Continued.

MS-MARS	*Lr24/Sr24*	*Sr38/Lr37/Yr17*	*Sr31/Lr26/Yr9*	*Sr24*-complex &*Sr31*-complex	*Sr24*-complex &*Sr38*-complex	*Sr31*-complex &*Sr38*-complex	*Sr24*-complex, *Sr38*-complex &*Sr31*-	None
21	■	-	■	■	-	-	-	-
22	■	■	-	-	■	-	-	-
23	■	■	-	-	■	-	-	-
24	-	■	■	-	-	■	-	-
25	■	-	■	■	-	-	-	-
26	-	■	-	-	-	-	-	-
27	■	-	■	■	-	-	-	-
28	-	-	■	-	-	-	-	-
29	■	■	-	-	■	-	-	-
30	■	-	■	■	-	-	-	-
31	■	-	■	■	-	-	-	-
32	-	-	■	-	-	-	-	-
33	■	-	-	-	-	-	-	-
34	■	-	■	■	-	-	-	-
35	-	-	-	-	-	-	-	■
36	■	-	■	■	-	-	-	-
37	■	-	■	■	-	-	-	-
38	-	-	-	-	-	-	-	■
39	■	-	-	-	-	-	-	-
40	■	-	■	■	-	-	-	-
41	■	-	■	■	-	-	-	-
42	-	-	-	-	-	-	-	■
43	■	-	■	■	-	-	-	-
44	-	-	■	-	-	-	-	-
45	■	■	■	■	■	■	■	-

Table 7.1. Continued.

MS-MARS	*Lr24*/*Sr24*	*Sr38*/*Lr37*/*Yr17*	*Sr31*/*Lr26*/*Yr9*	*Sr24*-complex &*Sr31*-complex	*Sr24*-complex &*Sr38*-complex	*Sr31*-complex &*Sr38*-complex	*Sr24*-complex, *Sr38*-complex &*Sr31*-	None
46		-			-	-	-	-
47		-			-	-	-	-
48	-	-		-	-	-	-	-
49		-			-	-	-	-
50			-	-		-	-	-
51		-			-	-	-	-
52		-			-	-	-	-
53		-	-	-	-	-	-	-
54			-	-		-	-	-
55		-			-	-	-	-
56		-	-	-	-	-	-	-
57		-			-	-	-	-
58		-			-	-	-	-
59		-			-	-	-	-
60		-			-	-	-	-
61	-		-	-	-	-	-	-
62								-
63	-	-		-	-	-	-	-
64								-
SUM	**49**	**15**	**41**	**33**	**11**	**6**	**5**	**4**
FREQUENC	**77%**	**23%**	**64%**	**52%**	**17%**	**9%**	**8%**	**6%**

Table 7.2. Frequencies of rust resistance gene complexes in the 64 MS-MARS lines sourced from Marais & Botes (2003).

Complex/es	**n**	**%**
Sr31/*Lr26*/*Yr9*	41	77%
Lr24/*Sr24*	49	23%
Lr37/*Sr38*/*Yr17*	15	64%
Lr24/*Sr24* with *Sr31*/*Lr26*/*Yr9*	33	52%
Lr37/*Sr38*/*Yr17* with *Lr24*/*Sr24*	11	17%
Lr37/*Sr38*/*Yr17* with *Sr31*/*Lr26*/*Yr9*	6	9%
Sr31/*Lr26*/*Yr9* with *Lr24*/*Sr24* &*Lr37*/*Sr38*/*Yr17*	5	8%
None	4	6%

7.3. Addendum C: GLM and NNA ANOVA tables of all four locations generated by Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada)

Table 7.3. GLM ANOVA for Langgewens.

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	79	20.56			
BLOCK	3	2.86	0.95	6.37	0.0008
GENOTYPE	19	9.18	0.48	3.23	0.0003
ERROR	57	8.52	0.15		

Table 7.4. Descriptive statistics of Langgewens

Parameter	Value
Grand Mean	2.40 ton/ha
R^2	58.6%
h^2	35.8%
C.V.	16.2%
L.S.D. (5%)	0.46 ton/ha

Table 7.5. GLM ANOVA for Welgevallen.

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	79	19.60			
BLOCK	3	0.05	0.02	0.18	0.9118
GENOTYPE	19	14.03	0.74	7.63	0.0000
ERROR	57	5.52	0.10		

Table 7.6. Descriptive statistics of Welgevallen

Parameter	Value
Grand Mean	1.41 ton/ha
R^2	71.8%
h^2	62.4%
C.V.	22.1%
L.S.D. (5%)	0.37 ton/ha

Table 7.7. GLM ANOVA for Tygerhoek.

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	79	42.33			
BLOCK	3	1.80	0.60	3.12	0.0296
GENOTYPE	19	29.86	1.57	8.40	0.0000
ERROR	57	10.66	0.19		

Table 7.8. Descriptive statistics of Tygerhoek

Parameter	Value
Grand Mean	5.62 ton/ha
R^2	74.8%
h^2	64.9%
C.V.	7.69%
L.S.D. (5%)	0.51 ton/ha

Table 7.9. GLM ANOVA for Hartsvallei.

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	59	45.11			
BLOCK	2	1.51	0.76	4.13	0.0239
GENOTYPE	19	36.63	1.93	10.52	0.0000
ERROR	38	6.96	0.18		

Table 7.10. Descriptive statistics of Hartsvallei.

Parameter	Value
Grand Mean	7.86 ton/ha
R^2	84.6%
h^2	76.0%
C.V.	5.4%
L.S.D. (5%)	0.59 ton/ha

7.4. Addendum D: NNA ANOVA tables of all four locations generated by Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada)

Table 7.11. ANOVA from adjusted data obtained from NNA of Langgewens:

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	78	16.59			
BLOCK	3	0.73	0.24	1.68	0.1825
GENOTYPE	19	7.71	0.41	2.79	0.0015
ERROR	56	8.15	0.15		

Table 7.12. Descriptive statistics obtained from the NNA's adjusted data for Langgewens:

Parameter	Value
Grand Mean	2.39 ton/ha
R^2	50.9%
h^2	73.6%
C.V.	15.9%
L.S.D. (5%)	0.45 ton/ha

Table 7.13. ANOVA from adjusted data obtained from NNA of Welgevallen:

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	78	16.28			
BLOCK	3	0.03	0.01	0.13	0.9443
GENOTYPE	19	11.12	0.59	6.39	0.0000
ERROR	56	5.13	0.09		

Table 7.14. Descriptive statistics obtained from the NNA's adjusted data for Welgevallen:

Parameter	Value
Grand Mean	1.41 ton/ha
R^2	68.5%
h^2	86.5%
C.V.	21.4%
L.S.D. (5%)	0.36 ton/ha

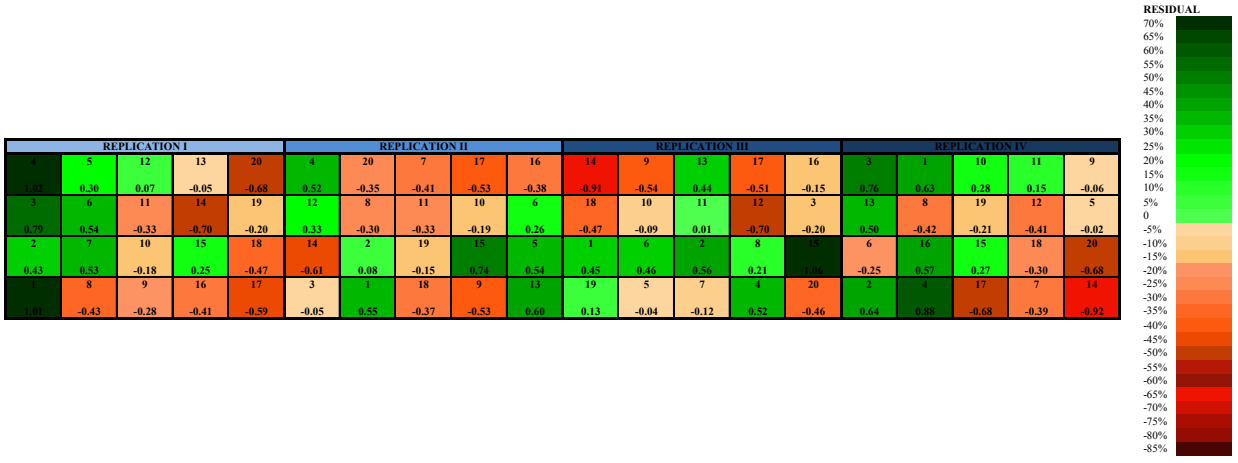
Table 7.15. ANOVA from adjusted data obtained from NNA of Tygerhoek:

SOURCE	DF	SS	MS	F-VALUE	P-VALUE
TOTAL	78	39.41			
BLOCK	3	0.35	0.12	0.70	0.5588
GENOTYPE	19	29.60	1.56	9.23	0.0000
ERROR	56	9.46	0.17		

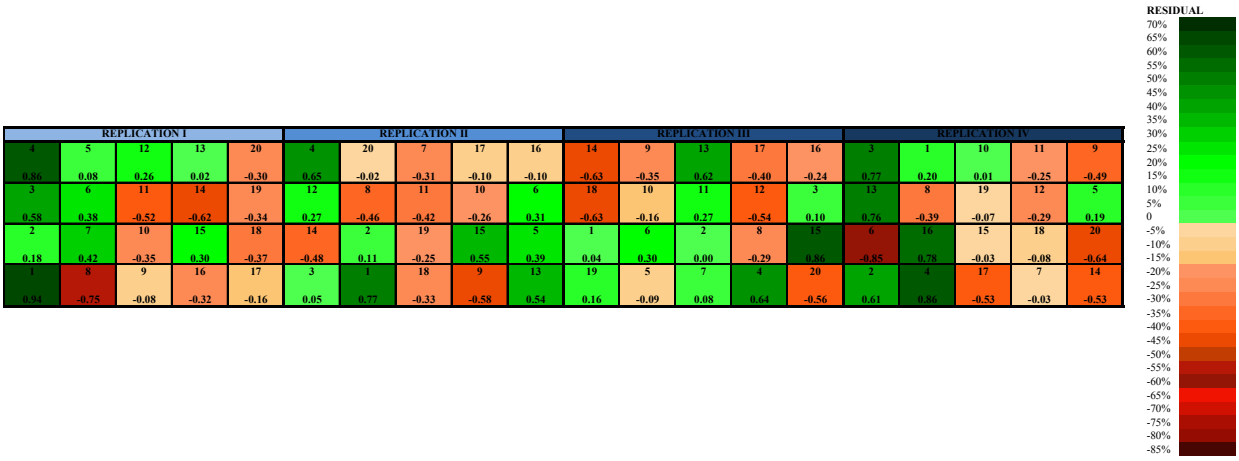
Table 7.16. Descriptive statistics obtained from the NNA's adjusted data for Tygerhoek:

Parameter	Value
Grand Mean	5.62 ton/ha
R^2	76.0%
h^2	90.2%
C.V.	7.3%
L.S.D. (5%)	0.49 ton/ha

7.5. Addendum E: Each plot residual plotted out on Welgevallen, Tygerhoek and Hartsvallei's RCBD grid.



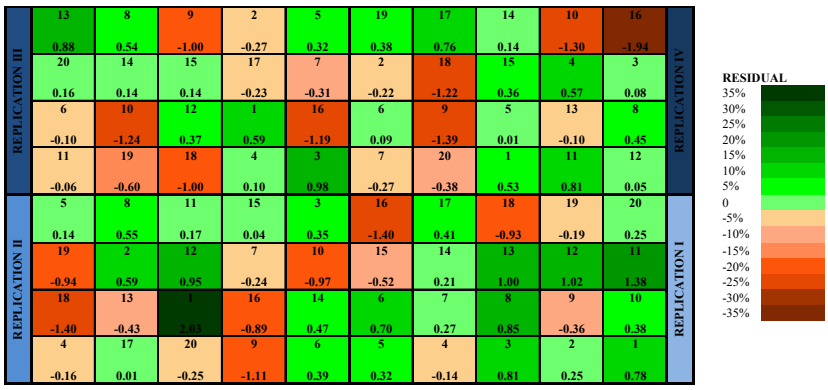
a.



b.

Figure 7.8. a). Unadjusted plot residuals, ascertained from the raw data, plotted on to the experimental layout of the Welgevallen experiment. Unlike the Langgewens grid layout, no large clear trend can be seen. In accordance to the NNA weight small East-to-West trends did exist, although not as prominent as with the Langgewens experiment. b). Adjusted plot residuals, ascertained from the adjusted NNA data, plotted on to the experimental layout of the Welgevallen experiment.

a.



b.

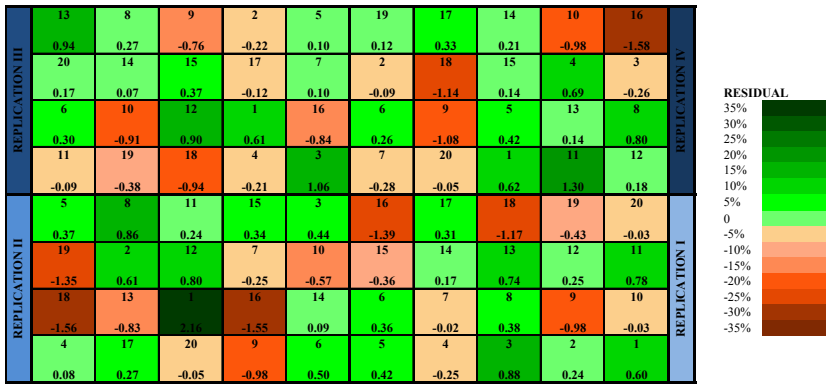


Figure 7.9. a). Unadjusted plot residuals, ascertained from the raw data, plotted on to the experimental layout of the Tygerhoek experiment. In accordance to the NNA, very small East-to-West trends did exist (NNA weight = 0.105). The CV, which is a measure of homogeneity between the replications, were very small (CV = 7.69%). Due to the homogeneity of the trial the East-to-West trends were also small. No large field trends could have been seen from the unadjusted residual grid layout. b). Adjusted plot residuals, ascertained from the adjusted NNA data, plotted on to the experimental layout of the Tygerhoek experiment.

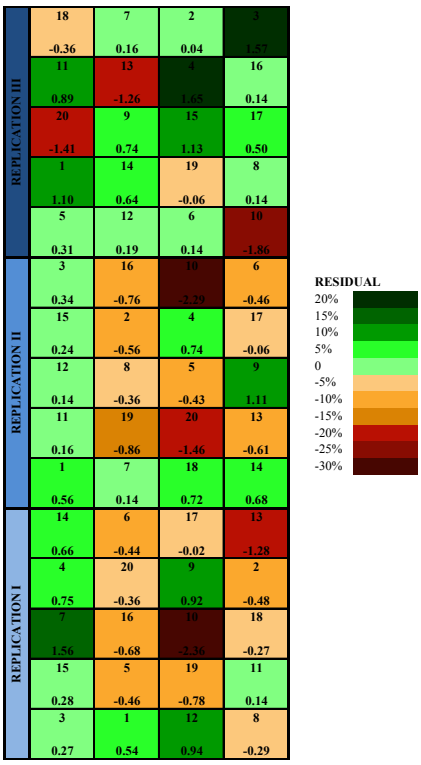


Figure 7.10. Unadjusted plot residuals, ascertained from the raw data, plotted on to the experimental layout of the Hartsvallei experiment. Due to a very homogenous soil grid, the NNA did not pick up any East-to-West field trends and thus did not perform any data adjustments through the NNA.

7.6. Addendum F: AMMI of all four locations generated by Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada)

Table 7.17. AMMI analysis of all four experiments (Langgewens, Welgevallen, Tygerhoek and Hartsvallei):

SOURCE	DF	SS	MS	% of total variance	% of GEI variance	P-VALUE
TOTAL	239	1706.49				
ENVIRONMENTS	3	1574.32	524.77	92.3%		0.0000
REPS WITHIN ENV.	8	1.69	0.21			
GENOTYPE	19	23.99	1.26	1.4%		0.0001
GEN. X ENV.	57	45.52	0.80	2.7%		0.0004
IPCA 1	21	26.99	1.29		59.3%	0.0001
IPCA 2	19	14.74	0.78		32.4%	0.0004
IPCA 3	17	3.79	0.22		8.3%	0.0010
ERROR	152	15.45	0.10			

Table 7.18. Descriptive statistics obtained from the AMMI analysis:

Parameter	Value
Grand Mean	4.32 ton/ha
R^2	99.1%
C.V.	7.4%

Table 7.19. Genotypic yield and IPCA 1 scores of each entry plotted in the AMMI bi-plot:

Name	Yield	IPCA 1 score
SST 056	4.87	0.15
SST 047	4.28	-0.16
SST 806	4.67	0.31
SST 867	4.83	0.37
US 1010	4.26	-0.03
MS-MARS-06	4.29	-0.11
MS-MARS-07	4.48	0.31
MS-MARS-08	4.14	0.03
MS-MARS-09	4.19	0.55
MS-MARS-10	3.60	-0.67
MS-MARS-11	4.53	0.12
MS-MARS-12	4.47	0.22
MS-MARS-13	4.40	-0.49
MS-MARS-14	4.48	0.32
MS-MARS-15	4.71	0.09
MS-MARS-16	3.91	-0.06
MS-MARS-17	4.30	0.09
MS-MARS-18	3.96	0.21
MS-MARS-19	3.99	-0.17
MS-MARS-20	4.10	-0.42

Table 7.20. Average yield and IPCA 1 scores of each location plotted on the AMMI bi-plot:

Location	Yield	IPCA 1 score
Hartsvallei	7.86	1.49
Langgewens	2.42	-0.67
Tygerhoek	5.60	-0.39
Welgevallen	1.42	-0.44

7.7. Addendum G. Quality characteristics tables. Quality for each line evaluated on grounds of number of quality characteristic deviations from the quality standard SST806. Method used by SAGL.

Table 7.21. Quality of the biological standard, SST806, against whom each of the fifteen MS-MARS lines were compared to.

SST806			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
3	TYGERHOEK	81.5	79.0	36.4	2.4	448	14.4	51.1	10.8	3.2	59.3	757
3	WELGEVALLEN	81.2	73.0	35.3	2.4	433	16.3	55.0	11.0	3.3	59.6	770
3	LANGGEWENS	79.3	68.1	36.7	2.5	422	18.0	55.1	11.3	3.3	60.1	753
3	HARTSVALLEI	81.0	64.5	41.0	2.6	398	12.8	51.4	10.9	2.1	60.1	803
	AVERAGE	80.7	71.1	37.3	2.4	425	15.4	53.2	11.0	3.0	59.8	771
	SD	0.96	6.28	2.49	0.12	21.34	2.26	2.22	0.21	0.57	0.39	22.83
	CV	1%	10%	9%	8%	17%	3%	1%	5%	24%	1%	4%

Table 7.22. Quality of SST056 compared to SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

SST056			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
1	TYGERHOEK	79.3	71.9	39.1	2.4	416	14.9	52.0	11.9	2.7	59.6	763.3
1	WELGEVALLEN	78.7	68.0	38.9	2.5	400	15.6	50.9	11.7	2.6	60.7	766.7
1	LANGGEWENS	76.7	68.9	32.8	2.2	425	15.6	51.7	11.4	3.3	61.2	720.0
1	HARTSVALLEI	78.3	56.4	41.2	2.7	288	14.8	52.3	10.5	1.8	59.5	796.7
AVERAGE		78.3	66.3	38.0	2.5	382	15.2	51.7	11.3	2.6	60.3	762
SD		1.11	6.82	3.60	0.19	63.95	0.43	0.59	0.60	0.62	0.82	31.56
CV		1%	10%	9%	8%	17%	3%	1%	5%	24%	1%	4%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-2.5	-4.9	0.6	0.0%	-10%	-0.1%	-1.4%	0.4%	-12.6%	0.5	-1%

Table 7.23. Quality of SST047 compared to SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

SST047			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
2	TYGERHOEK	81.6	65.9	41.7	2.6	451	17.9	54.2	13.6	2.8	63.4	806.7
2	WELGEVALLEN	80.9	73.5	35.3	2.4	434	18.1	56.2	12.9	2.8	62.5	781.7
2	LANGGEWENS	79.0	63.7	38.2	2.5	408	17.4	55.2	11.5	2.8	60.5	700.0
2	HARTSVALLEI	79.1	58.0	44.2	2.9	359	15.3	50.8	12.9	2.1	63.4	893.3
AVERAGE		80.1	65.3	39.8	2.6	413	17.2	54.1	12.7	2.6	62.4	795
SD		1.28	6.41	3.89	0.22	39.92	1.32	2.38	0.88	0.36	1.38	79.60
CV		2%	10%	10%	8%	10%	8%	4%	7%	14%	2%	10%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-0.6	-5.9	2.5	0.2%	-3%	1.8%	1.0%	1.7%	-12.0%	2.6	3%

Table 7.24. Quality of SST867 compared to SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

SST867			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
4	TYGERHOEK	79.4	75.0	32.3	2.2	415	16.5	52.4	11.7	3.0	60.7	782.5
4	WELGEVALLEN	81.7	72.3	30.9	2.1	382	16.8	54.5	9.7	4.2	57.9	725.0
4	LANGGEWENS	80.3	64.6	35.1	2.5	431	17.4	56.0	11.1	3.9	59.9	715.0
4	HARTSVALLEI	81.1	64.3	39.7	2.6	277	14.8	56.6	10.4	2.2	59.5	796.7
AVERAGE		80.6	69.0	34.5	2.4	376	16.4	54.9	10.7	3.3	59.5	755
SD		1.01	5.45	3.85	0.22	69.32	1.12	1.89	0.85	0.89	1.17	40.79
CV		1%	8%	11%	9%	18%	7%	3%	8%	27%	2%	5%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-0.1	-2.1	-2.8	-0.1%	-12%	1.0%	1.7%	-0.3%	11.6%	-0.3	-2%

Table 7.25. Quality of US1010 compared to SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

US1010			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
5	TYGERHOEK	80.8	68.7	44.5	2.7	445	14.7	51.6	11.7	3.2	60.5	738.3
5	WELGEVALLEN	80.2	73.1	40.7	2.6	392	15.0	52.3	11.8	3.7	60.8	723.3
5	LANGGEWENS	79.2	66.7	38.9	2.5	420	16.3	53.8	11.2	4.0	60.0	685.0
5	HARTSVALLEI	78.9	66.3	48.6	3.1	343	12.1	54.9	12.1	2.5	62.0	811.7
AVERAGE		79.8	68.7	43.2	2.7	400	14.5	53.1	11.7	3.3	60.8	740
SD		0.86	3.15	4.33	0.25	43.83	1.77	1.46	0.38	0.67	0.87	53.04
CV		1%	5%	10%	9%	11%	12%	3%	3%	20%	1%	7%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.0	-2.4	5.8	0.3%	-6%	-0.8%	0.0%	0.7%	11.8%	1.0	-4%

Table 7.26. Quality of each of MS-MARS-06 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-06			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
6	TYGERHOEK	80.6	71.1	39.9	2.5	402	15.0	49.1	11.3	2.4	60.1	690.0
6	WELGEVALLEN	79.7	75.4	34.1	2.3	241	17.4	52.4	11.9	2.7	61.1	711.7
6	LANGGEWENS	79.1	65.3	36.6	2.5	429	17.3	52.6	10.6	2.9	60.0	668.3
6	HARTSVALLEI	78.6	65.8	44.9	2.8	272	14.2	52.3	11.6	1.7	61.4	728.3
	AVERAGE	79.5	69.4	38.9	2.5	336	16.0	51.6	11.4	2.4	60.7	700
	SD	0.86	4.81	4.65	0.20	93.17	1.60	1.64	0.58	0.54	0.70	26.08
	CV	1%	7%	12%	8%	28%	10%	3%	5%	22%	1%	4%
	LOWER TOLERANCE LEVEL	-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
	UPPER TOLERANCE LEVEL	-	-	4	-	-	5%	-	-	15%	2	-
	DEVIATION	-1.2	-1.7	1.5	0.1%	-21%	0.6%	-1.6%	0.4%	-18.0%	0.9	-9%

Table 7.27. Quality of each of MS-MARS-07 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-07			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
7	TYGERHOEK	79.1	74.0	35.5	2.4	402	14.8	49.0	12.4	2.0	60.8	738.3
7	WELGEVALLEN	79.2	75.6	32.5	2.4	273	15.7	51.2	12.1	2.1	61.4	773.3
7	LANGGEWENS	77.8	71.1	35.1	2.4	430	16.4	51.0	11.1	2.8	60.5	688.3
7	HARTSVALLEI	77.9	69.2	38.9	2.7	266	13.4	50.1	10.4	1.4	59.5	650.0
	AVERAGE	78.5	72.5	35.5	2.4	343	15.1	50.3	11.5	2.1	60.5	713
	SD	0.74	2.86	2.67	0.15	85.59	1.31	1.02	0.91	0.57	0.78	54.34
	CV	1%	4%	8%	6%	25%	9%	2%	8%	27%	1%	8%
	LOWER TOLERANCE LEVEL	-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
	UPPER TOLERANCE LEVEL	-	-	4	-	-	5%	-	-	15%	2	-
	DEVIATION	-2.3	1.3	-1.9	0.0%	-19%	-0.2%	-2.8%	0.5%	-30.2%	0.7	-8%

Table 7.28. Quality of each of MS-MARS-08 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-08			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
8	TYGERHOEK	80.3	74.8	38.6	2.5	416	13.9	54.0	11.7	2.1	60.7	688.3
8	WELGEVALLEN	80.1	74.3	35.0	2.5	356	15.0	53.4	11.9	2.3	61.0	735.0
8	LANGGEWENS	79.4	66.7	37.1	2.4	438	16.8	53.3	11.6	3.4	60.6	687.5
8	HARTSVALLEI	77.5	68.3	44.2	2.8	280	12.0	49.5	11.0	1.5	60.4	650.0
AVERAGE		79.3	71.0	38.7	2.5	373	14.4	52.5	11.6	2.3	60.7	690
SD		1.27	4.11	3.92	0.20	70.84	2.01	2.08	0.40	0.78	0.26	34.80
CV		2%	6%	10%	8%	19%	14%	4%	3%	34%	0%	5%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.4	-0.1	1.4	0.1%	-12%	-0.9%	-0.6%	0.6%	-22.2%	0.9	-10%

Table 7.29. Quality of each of MS-MARS-09 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-09			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
9	TYGERHOEK	75.2	69.3	37.7	2.4	409	17.4	57.2	12.0	2.9	61.0	796.7
9	WELGEVALLEN	77.6	68.8	36.4	2.5	385	15.7	54.5	13.3	4.3	62.3	795.0
9	LANGGEWENS	75.2	63.3	33.2	2.3	438	17.8	53.6	12.3	3.3	61.6	795.0
9	HARTSVALLEI	75.2	62.5	46.7	2.9	288	14.3	54.4	10.9	2.6	60.1	718.3
AVERAGE		75.8	66.0	38.5	2.5	380	16.3	54.9	12.1	3.3	61.3	776
SD		1.20	3.55	5.78	0.26	65.21	1.62	1.58	0.99	0.76	0.93	38.62
CV		2%	5%	15%	10%	17%	10%	3%	8%	23%	2%	5%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-4.9	-5.2	1.2	0.1%	-11%	0.9%	1.8%	1.1%	9.6%	1.5	1%

Table 7.30. Quality of each of MS-MARS-10 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-10			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
10	TYGERHOEK	79.4	82.6	33.7	2.3	377.7	12.9	48.2	10.8	2.4	59.4	743.3
10	WELGEVALLEN	79.8	81.5	33.0	2.4	309.3	14.3	47.1	11.5	2.6	60.4	750.0
10	LANGGEWENS	77.6	66.8	33.6	2.3	438.3	16.4	51.8	11.5	2.7	60.4	701.7
10	HARTSVALLEI	77.2	70.8	41.9	2.9	278.7	12.6	46.2	10.8	2.1	60.1	690.0
AVERAGE		78.5	75.4	35.6	2.5	351	14.1	48.3	11.2	2.4	60.1	721
SD		1.29	7.83	4.25	0.26	71.43	1.74	2.49	0.39	0.30	0.48	29.86
CV		2%	10%	12%	10%	20%	12%	5%	4%	12%	1%	4%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-2.2	4.3	-1.8	0.0%	-17%	-1.3%	-4.8%	0.2%	-17.8%	0.3	-6%

Table 7.31. Quality of each of MS-MARS-11 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-11			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
11	TYGERHOEK	78.3	66.3	41.8	2.5	318.3	17.3	55.8	11.7	2.3	59.7	680.0
11	WELGEVALLEN	79.8	66.6	32.9	2.3	349.5	17.5	53.3	11.9	2.5	61.0	735.0
11	LANGGEWENS	77.5	68.1	30.6	2.2	444.3	17.5	51.8	11.5	3.3	60.5	741.7
11	HARTSVALLEI	79.2	62.3	41.8	2.7	271.7	16.9	58.8	10.5	1.9	59.6	706.7
AVERAGE		78.7	65.8	36.8	2.5	346	17.3	54.9	11.4	2.5	60.2	716
SD		1.03	2.47	5.86	0.23	72.97	0.28	3.06	0.62	0.60	0.66	28.30
CV		1%	4%	16%	9%	21%	2%	6%	5%	24%	1%	4%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-2.0	-5.3	-0.6	0.0%	-19%	1.9%	1.8%	0.4%	-16.1%	0.4	-7%

Table 7.32. Quality of each of MS-MARS-12 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-12			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
12	TYGERHOEK	82.6	76.7	40.3	2.5	350.3	13.1	50.9	11.1	1.8	59.3	671.7
12	WELGEVALLEN	82.7	75.7	39.8	2.6	280.0	14.5	48.7	10.9	1.9	59.6	676.7
12	LANGGEWENS	79.9	67.9	38.9	2.5	414.3	15.8	52.8	10.5	2.6	59.1	705.0
12	HARTSVALLEI	78.8	66.5	43.2	2.7	272.7	13.4	50.7	10.3	1.5	59.2	646.7
AVERAGE		81.0	71.7	40.5	2.6	329	14.2	50.8	10.7	2.0	59.3	675
SD		1.96	5.23	1.84	0.10	66.61	1.20	1.67	0.37	0.49	0.22	23.92
CV		2%	7%	5%	4%	20%	8%	3%	3%	25%	0%	4%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		0.3	0.6	3.2	0.1%	-23%	-1.2%	-2.4%	-0.3%	-34.4%	-0.5	-12%

Table 7.33. Quality of each of MS-MARS-13 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue. Where certain industrial quality characteristics were significantly better than SST806, it is highlighted in light green.

MS-MARS-13			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
13	TYGERHOEK	81.0	61.5	42.4	2.7	388.0	18.1	57.1	13.5	3.5	63.2	693.3
13	WELGEVALLEN	79.8	65.2	37.4	2.6	276.0	17.9	55.6	14.0	4.4	64.1	761.7
13	LANGGEWENS	78.6	65.5	36.6	2.4	435.3	17.4	55.7	11.8	3.1	60.9	676.7
13	HARTSVALLEI	76.9	56.1	40.6	2.7	251.5	16.0	57.8	12.4	2.4	62.4	805.0
AVERAGE		79.1	62.1	39.3	2.6	338	17.4	56.5	12.9	3.4	62.7	734
SD		1.76	4.34	2.71	0.17	88.13	0.96	1.09	1.03	0.86	1.39	59.85
CV		2%	7%	7%	6%	26%	6%	2%	8%	26%	2%	8%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.7	-9.1	1.9	0.2%	-21%	2.0%	3.4%	1.9%	12.9%	2.9	-5%

Table 7.34. Quality of each of MS-MARS-14 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-14			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
14	TYGERHOEK	80.3	81.2	38.7	2.5	410.0	13.8	49.5	10.4	2.0	59.6	680.0
14	WELGEVALLEN	81.0	81.9	35.8	2.5	364.3	12.9	45.0	12.2	2.2	61.4	712.5
14	LANGGEWENS	78.3	67.5	34.6	2.3	430.7	16.6	52.6	12.0	2.5	59.8	688.3
14	HARTSVALLEI	77.6	71.1	43.9	2.9	270.0	11.5	44.8	10.3	1.6	59.2	647.5
AVERAGE		79.3	75.4	38.2	2.6	369	13.7	48.0	11.2	2.0	60.0	682
SD		1.62	7.21	4.14	0.23	71.43	2.17	3.77	1.04	0.37	0.99	26.86
CV		2%	10%	11%	9%	19%	16%	8%	9%	18%	2%	4%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.4	4.3	0.9	0.1%	-13%	-1.7%	-5.2%	0.2%	-31.2%	0.2	-12%

Table 7.35. Quality of each of MS-MARS-15 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-15			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
15	TYGERHOEK	80.3	72.3	39.8	2.5	403.3	16.3	53.5	10.7	2.2	61.1	713.3
15	WELGEVALLEN	79.5	67.7	39.1	2.6	373.7	16.9	52.6	11.0	2.2	59.6	731.7
15	LANGGEWENS	79.2	65.9	36.9	2.4	430.0	17.4	54.3	11.4	3.3	59.2	693.3
15	HARTSVALLEI	77.4	58.3	47.9	2.9	277.7	16.0	55.7	10.4	1.9	59.4	705.0
AVERAGE		79.1	66.1	40.9	2.6	371	16.6	54.0	10.8	2.4	59.9	711
SD		1.21	5.83	4.81	0.23	66.44	0.62	1.35	0.42	0.64	0.87	16.13
CV		2%	9%	12%	9%	18%	4%	2%	4%	27%	1%	2%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.6	-5.1	3.6	0.2%	-13%	1.3%	0.9%	-0.1%	-19.7%	0.1	-8%

Table 7.36. Quality of each of MS-MARS-16 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-16			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
16	TYGERHOEK	80.4	68.6	40.0	2.5	402.7	16.0	53.6	11.6	3.4	60.6	656.7
16	WELGEVALLEN	78.6	66.6	35.6	2.5	278.0	18.7	57.8	12.8	4.5	62.3	708.3
16	LANGGEWENS	78.7	63.1	37.9	2.5	405.7	17.7	53.8	11.1	2.9	59.8	746.7
16	HARTSVALLEI	78.2	51.3	46.5	2.9	283.7	15.5	55.8	11.5	2.8	61.1	765.0
AVERAGE		79.0	62.4	40.0	2.6	343	17.0	55.2	11.8	3.4	60.9	719
SD		0.99	7.72	4.72	0.22	71.25	1.49	2.01	0.75	0.75	1.07	47.89
CV		1%	12%	12%	8%	21%	9%	4%	6%	22%	2%	7%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.8	-8.7	2.7	0.1%	-19%	1.6%	2.1%	0.8%	14.1%	1.1	-7%

Table 7.37. Quality of each of MS-MARS-17 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-17			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
17	TYGERHOEK	78.7	85.8	35.5	2.4	423.0	13.7	48.6	11.1	3.7	59.9	766.7
17	WELGEVALLEN	79.0	84.0	32.5	2.4	347.0	15.3	51.9	11.9	4.5	61.0	723.3
17	LANGGEWENS	78.1	65.3	37.7	2.5	421.0	17.8	54.6	11.5	3.5	60.4	740.0
17	HARTSVALLEI	77.4	66.4	42.0	2.9	284.7	14.2	52.3	10.2	3.5	59.2	721.7
AVERAGE		78.3	75.4	36.9	2.5	369	15.3	51.8	11.2	3.8	60.1	738
SD		0.68	11.03	4.00	0.21	66.37	1.83	2.47	0.71	0.51	0.77	20.88
CV		1%	15%	11%	8%	18%	12%	5%	6%	13%	1%	3%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-2.4	4.2	-0.5	0.1%	-13%	-0.1%	-1.3%	0.2%	27.4%	0.3	-4%

Table 7.38. Quality of each of MS-MARS-18 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-18			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
18	TYGERHOEK	81.2	81.9	38.2	2.5	429.7	12.3	48.1	11.6	2.6	60.4	700.0
18	WELGEVALLEN	80.3	82.3	32.8	2.4	316.7	13.0	45.8	12.4	2.5	61.8	742.5
18	LANGGEWENS	78.3	64.5	37.0	2.4	442.7	16.0	51.0	11.6	3.1	60.6	665.0
18	HARTSVALLEI	78.1	66.3	42.3	2.9	266.3	12.8	47.2	10.7	2.1	59.9	717.5
AVERAGE		79.5	73.8	37.6	2.6	364	13.5	48.0	11.5	2.6	60.7	706
SD		1.51	9.66	3.90	0.23	86.18	1.68	2.20	0.69	0.42	0.80	32.56
CV		2%	13%	10%	9%	24%	12%	5%	6%	16%	1%	5%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-1.3	2.6	0.3	0.1%	-14%	-1.8%	-5.1%	0.6%	-13.6%	0.9	-8%

Table 7.39. Quality of each of MS-MARS-19 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-19			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
19	TYGERHOEK	81.0	68.0	43.0	2.6	441.7	16.0	51.8	12.1	3.7	61.2	725.0
19	WELGEVALLEN	82.6	69.1	38.9	2.6	285.0	16.4	54.4	10.8	3.9	59.4	695.0
19	LANGGEWENS	78.0	64.3	35.9	2.4	442.0	16.9	52.7	12.1	4.1	61.4	725.0
19	HARTSVALLEI	79.5	63.3	43.8	2.8	344.3	15.3	54.8	10.9	3.1	60.3	748.3
AVERAGE		80.3	66.2	40.4	2.6	378	16.1	53.4	11.5	3.7	60.6	723
SD		1.99	2.83	3.70	0.16	77.31	0.68	1.40	0.73	0.44	0.92	21.86
CV		2%	4%	9%	6%	20%	4%	3%	6%	12%	2%	3%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-0.5	-5.0	3.1	0.1%	-11%	0.8%	0.3%	0.5%	24.2%	0.8	-6%

Table 7.40. Quality of each of MS-MARS-20 showing each quality characteristic's deviation from SST806. When it deviated more than the tolerable ranges, the deviation value is highlighted by light blue.

MS-MARS-20			SKCS				CD1		FLOUR	MIXOGRAPH		BAKING
ENTRY	LOCATION	HLM	HI	TKM	DIAM	FN	BFY	EX	PROT	PT	ABS	VOL
20	TYGERHOEK	79.2	73.6	37.5	2.5	442.0	18.0	54.1	11.3	2.2	60.0	750.0
20	WELGEVALLEN	78.1	71.5	35.0	2.4	405.7	17.4	53.2	11.7	2.5	60.6	763.3
20	LANGGEWENS	77.6	64.1	34.8	2.4	449.7	18.0	53.4	12.7	3.0	62.2	745.0
20	HARTSVALLEI	77.1	59.3	47.5	3.0	369.7	17.0	55.2	10.3	2.4	59.2	711.7
AVERAGE		78.0	67.1	38.7	2.6	417	17.6	54.0	11.5	2.5	60.5	743
SD		0.88	6.60	6.02	0.29	36.79	0.49	0.89	1.00	0.35	1.26	21.96
CV		1%	10%	16%	11%	9%	3%	2%	9%	14%	2%	3%
LOWER TOLERANCE LEVEL		-1.8	-	-4	-	-15%	-5%	-1.5%	-1%	-25%	-2	-10%
UPPER TOLERANCE LEVEL		-	-	4	-	-	5%	-	-	15%	2	-
DEVIATION		-2.7	-4.0	1.3	0.1%	-2%	2.3%	0.8%	0.5%	-14.6%	0.7	-4%

7.8. Addendum H. The rust scores taken in 2011 and 2012 at Makhathini and Bethlehem.

Table 7.41. Individual rust scores taken in 2011 and 2012 at Makhathini and Bethlehem of each entry.

		MAKHATHINI 2011		MAKHATHINI 2012		BETHLEHEM 2011
				DISEASE		
		STEM RUST (SR)	LEAF RUST (LR)	STEM RUST (SR)	LEAF RUST (LR)	STRIPE RUST (YR)
				PATHOTYPES		
ENTRY	NAME	UVPgt60	UVPrt9 &UVPrt13	UVPgt60	UVPrt9 &UVPrt13	6E22A-
SPREADER	MOROCCO	40S	60S	60S	80S	80S
1	SST056	10MR	10MR	5R-MR	10MR	20MS
2	SST047	10MR	10MR	5R-MR	10MS	10MS
3	SST806	20MS	20MS	20S	40S	10MS
4	SST867	20S	20S	20S	30S	5R-MR
5	US1010	-	-	5R-MR	10MR	-
6	MS-MARS-06	20S	20S	30S	40S	20MS
7	MS-MARS-07	5R-MR	10MR	5R-MR	5R-MR	10MS
8	MS-MARS-08	10MR	10MR	10MR	10MR	tMS
9	MS-MARS-09	5R-MR	5R-MR	5R-MR	10MR	30S
10	MS-MARS-10	10MR	10MS	10MR	20MS	tMS
11	MS-MARS-11	10MS	10MS	5MS	20MS	tMS
12	MS-MARS-12	10MR	10MS	10MR	5MS	R
13	MS-MARS-13	10MS	20MS	5MS	10MS	R
14	MS-MARS-14	5R-MR	10MR	R	5MS	R
15	MS-MARS-15	10MR	10MR	5R-MR	5R-MR	10MS
16	MS-MARS-16	10MS	10MR	5MS	5R-MR	20MS
17	MS-MARS-17	10MR	10MR	10MS	10S	tMS
18	MS-MARS-18	5MS	10MS	20MS	5MS	R
19	MS-MARS-19	5R-MR	5R-MR	20S	20S	R
20	MS-MARS-20	10MS	10MR	10S	5MS	R